ORIGINAL ARTICLE

Formulation, Development, and *In Vitro*, *In Vivo, Ex Vivo* Characterization of *In Situ* Gel Containing Blueberry Extract for the Management of Glaucoma

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Abstract

Aim: This study focused on the formulation and evaluation of ocular *in situ* gels containing blueberry extract for potential therapeutic applications. **Materials and Methods:** Various parameters were assessed to determine the physical characteristics, stability, and performance of the formulated gels. These parameters included clarity, transparency, pH, viscosity, gelling capacity, *in vitro* and ex vivo drug release, sterility, ocular irritation, intraocular pressure (IOP) reduction, and pharmacokinetic profiles. Total number of six formulations were prepared and evaluated for different parameters. Percentage drug release of all the formulations were carried out by modified Franz diffusion cell using simulated tear fluid. **Results and Discussion:** B6 formulation has shown maximum percentage release of 98.35% in 8 h. *Ex vivo* studies indicated that 80.23% of the drug permeated from the formulations, demonstrating a sustained effect. Furthermore, *in situ* gel of blueberry extract (B6) demonstrated a good percentage reduction in IOP, with a value of 29.96 ± 2.88 % mmHg. The *in situ* gel formulation achieved a C_{max} of 10.5 ± 1.28 mg/mL after 4 h, which was approximately 10 times higher than the C_{max} of the timolol eye drop (0.908 mg/mL) obtained after 2 h. **Conclusion:** The results indicated that the *in situ* gels demonstrated favorable physical properties, stable pH, excellent gelling capacity, sustained drug release, and sterility. Furthermore, the gels exhibited no ocular irritation and effectively reduced IOP, offering potential benefits for glaucoma treatment. Pharmacokinetic analysis revealed enhanced drug bioavailability with the *in situ* gel formulations compared to conventional eye drops. Stability studies confirmed the formulation's robustness under various environmental conditions. These findings highlight the suitability of the *in situ* gel formulations for ocular drug delivery, emphasizing their potential as effective and safe therapeutic options.

Key words: Blueberry, glaucoma, *in vivo* pharmacokinetic studies, ocular drug delivery system

INTRODUCTION

Glaucoma is considered as a leading cause of initial blindness in the geriatric population as it primarily affects the optic nerve of eye and if it is not being treated at its detection, it can further lead to a severe form of blindness. Two forms of glaucoma have been found prominent which are the primary open-angle and closed angle glaucoma. In open-angle glaucoma, the trabecular meshwork which is situated near the cornea is associated with its fractional blockage which causes a less drainage of aqueous humor from eye resulting into an increased ocular pressure.[1,2] When the problem deceit in the iris of eye due to its outward expansion resulting into the partial obstruction of the drainage angle present between the iris and cornea, due to which the flow of intraocular fluid into the trabecular meshwork is reduced and thus the intraocular pressure is enhanced significantly. This is resulted into the formation of closed angle glaucoma.[3,4]

A key challenge frequently encountered during the development of ophthalmic delivery systems is the achievement of desired drug level at the target site, particularly within the anterior cavity of the eye, for sufficient time. This is mainly due to the complex anatomy and highly selective corneal barriers, which

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limit the entry of any exogenous substances to the ocular tissues.[5,6] Different types of ophthalmic vehicles such as eye drops, ointments, gels, and polymeric ocular inserts were developed in an attempt to enhance the pre-corneal residence time.[7] Among the various ophthalmic dosage forms evaluated so far, *in situ* gel drug delivery systems have been an extensive area of research during the past few decades. *In situ* gels are attractive since it can be suitably applied as drops or solutions into the conjunctival sac, wherein they undergo a phase conversion into a gel state upon exposure to either pH of the tear fluid, ocular surface temperature, or ions exists on the tear film.[8,9] Transition to gel state in the corneal surface extends the ocular residence resulting in better ocular bioavailability by minimizing rapid pre-corneal elimination, particularly due to nasolacrimal drainage and eye blinking.[10,11] It can also reduce the poor compliance due to frequent administration and risk of undesirable side effects associated with systemic drug absorption by virtue of pre-corneal elimination.[12] *In situ* activated transparent gel formulations are ideal for ocular therapy as it can be administered as liquid dosage form and avoids blurred vision. Besides, they exhibit excellent physicochemical characteristics such as bio adhesion, ocular tolerance, and sustained drug release properties than conventional ophthalmic preparations as a consequence of prolonged pre-corneal residence time. At present, these types of dosage forms are employed in many ocular conditions such as glaucoma, dry eye syndrome, age-related macular degeneration, and trachoma.[13,14]

Natural compounds such as blueberry extract have shown potential in reducing intraocular pressure (IOP) and protecting retinal cells. Blueberry extract, rich in anthocyanins and antioxidants, has demonstrated potential neuroprotective and IOP-lowering properties in pre-clinical studies.[15-17] In this research, we explore the development and evaluation of an *in situ* gel formulation containing blueberry extract as a novel approach for glaucoma management.

MATERIALS AND METHODS

Materials

In the present research study, various chemicals, solvents, and reagents were utilized for conducting experiments and analyses. The blueberry extract was purchased from Vihan Herbal and Food Ingredients, India. The polymers HPMCK4M, carbopol-934, sodium chloride, NaOH, and benzalkonium chloride (BKC) were purchased from HiMedia Laboratories, Mumbai, India. All the other chemicals, solvents, and reagents used were of analytical grade.

Methods

Preparation of pH-triggered in situ gel for blueberry extract

A solution of carbopol-934 with high concentration was prepared, and HPMC was subsequently introduced and allowed to undergo hydration. In the process of preparing blueberry extract with polymer solutions, the specified quantities of blueberry extract were introduced into the carbopol/HPMC solutions while maintaining a constant stirring motion until achieving complete homogeneity [Table 1]. BKC was subsequently introduced, and the solutions underwent filtration using a cellulose acetate membrane filter with a pore size of 0.2 mm. The solutions were subsequently diluted to a volume of 100 mL using distilled water and vigorously mixed at cold temperatures. All formulations underwent a 24-h equilibration period at room temperature before being assessed in both *in vitro* and *in vivo* experiments.[18-20]

Evaluation of in situ gels of blueberry extracts

Clarity and visual assessment

In this phase, we conducted a thorough visual examination of the physical appearance of all ocular *in situ* gels. This examination took place under fluorescent lighting and involved scrutinizing the formulations against both white and dark backgrounds. The primary focus was on assessing clarity, with a specific emphasis on detecting any presence of dirt, foreign particles, or insoluble materials within the formulations.[18,19]

pH measurement

The determination of pH values in the ocular *in situ* gel formulations involved utilizing a calibrated digital pH meter. To execute this process, the electrode of the calibrated pH meter was carefully submerged into the *in situ* gel sample. The reading was allowed to reach a state of equilibrium, and subsequently, the pH value was recorded. This step ensures accurate measurement and monitoring of the formulations' pH levels.[18,19]

Viscosity determination

Understanding the viscosity of the instilled formulation is critical for gauging the drug's residence time in the eye. As the concentration of polymers within the formulation increased, the viscosity also increased. To accurately calculate viscosity, a Brookfield Viscometer (specifically the DV-II + pro model with spindle no. 61) was employed. The viscosity assessment involved reversing the hierarchy of shear rate, and the average of two readings was considered. The pre-gelation viscosities of the formulations at pH 6 were evaluated by rotating the spindle at varying rpm settings, and viscosity readings were recorded accordingly.[18,19]

Gelling capacity evaluation

This step aimed to assess the *in situ* gel formulation's capability to undergo a sol-gel transition within the eye. To simulate this transition, simulated tear fluid (STF) was prepared to mimic the composition of natural tears. A drop of the *in situ* gel formulation was introduced into 2 mL of the prepared STF solution in a vial. The mixture was then placed in a temperature-controlled thermostat set at 37 ± 2°C,

Table 1: Formulation design of blueberry extract *in situ* gels

Ingredients	Formulation code					
	B1	B2	B3	B4	B5	B6
Blueberry extract (g)	0.05	0.05	0.05	0.05	0.05	0.05
HPMCK4M (g)	0.4	0.4	0.4	0.4	0.4	0.4
Carbopol-934 (g)	0.05	0.1	0.15	0.2	0.25	0.3
Sodium chloride (g)	0.9	0.9	0.9	0.9	0.9	0.9
0.1NNaOH (mL)	q.s	q.s	q.s	q.s	q.s	q.s
Benzalkonium chloride (%)	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water q.s (mL)	100	100	100	100	100	100

reflecting the physiological temperature of the eye. The duration required for gelation, indicating the transition from a liquid state to a gel-like viscosity, was recorded. In addition, the time taken for the gel to disintegrate, reverting to its liquid state, was also noted. This dual assessment provided insights into the *in situ* gel's ability to form and dissolve effectively.[18,19]

In vitro release studies

For this study, a modified Franz diffusion cell setup was employed. The *in situ* gel formulations were situated in the donor compartment, while freshly prepared STF occupied the receptor compartment. STF was prepared by dissolving sodium chloride (0.67 g), sodium bicarbonate (0.20 g), and calcium chloride dihydrate (0.008 g) in distilled water q. s. 100 mL. A dialysis membrane (mol. wt. 12000D), previously soaked in STF, separated the two compartments. At specified time intervals (0.5, 1, 2, 4, 6, and 8 h), 1 mL aliquots were withdrawn from the receptor compartment. After each withdrawal, 1 mL of fresh STF was replenished to maintain sink conditions. The withdrawn samples were then diluted with STF, filtered through 0.45 µm syringe filters, and subjected to high-performance liquid chromatography (HPLC) analysis to determine the concentrations of the extracted substances.[19,21,22]

Ex vivo drug permeation study

In this investigation, a modified Franz diffusion chamber was utilized, and rabbit corneal membranes served as the medium for studying drug permeability. Corneas were obtained from male New Zealand albino rabbits, transported under cold conditions, and immersed in normal saline solution at 4°C. A magnetic stirrer was used to maintain the permeation study at a temperature of 37 ± 0.2°C. The detached cornea was treated with 20 mL of *in situ* gels, and aliquots of the medium were extracted at specified time intervals (1, 2, 4, 6, and 8 h). The extracted aliquots were replaced with an equal volume of fresh media to ensure constant volume conditions. Subsequently, these samples underwent HPLC analysis to determine the concentration of the liberated extract.[19,22]

Sterility testing

The sterility testing process was crucial for ensuring the safety and efficacy of the ophthalmic product, specifically formulation B6. Aseptic procedures were employed in this evaluation, where the formulation was introduced into a medium containing soybean casein digest. This combination underwent incubation at a temperature of 35°C for a minimum of 14 days to facilitate potential bacterial proliferation. Throughout the incubation period, the sterility of the formulation was assessed by visually examining the transparency of the medium. The absence of observable proliferation in the culture medium during the 14-day monitoring period confirmed the sterility of the formulation, indicating its suitability for ocular administration without bacterial contamination.[23-25]

In vivo studies

Ocular irritation test

To evaluate the potential ocular irritation caused by the *in situ* gels, an ocular irritation assessment was conducted. This examination aimed to ascertain any adverse effects or discomfort on ocular tissues resulting from the interaction with the formulated substances. The significance of this assessment lies in determining the safety profile of the formulation.

The Draize test, recognized for its precision and reliability, was employed as the preferred method for assessing ocular irritancy. A group of seven male albino rabbits from New Zealand were chosen as test subjects to undergo the application of optimized *in situ* gel formulations.[26]

A measured quantity (50 µL) of the *in situ* gel was administered to the lower conjunctival sac of one eye for each rabbit, while the other eye remained untreated, serving as a control. Observations were conducted at specific intervals, including 1 h, 2 h, 4 h, and 8 h post-application. Any indications of ocular irritation, such as redness, swelling, discharge, or alterations in the cornea or conjunctiva, were meticulously documented.

The Draize scoring system was employed to assess the degree of ocular irritation. The observations were recorded, and scores were assigned based on the severity and reversibility of the observed effects. The accumulated scores were then analyzed to determine the ocular irritation potential of the *in situ* gels. The ranking, ranging from 0 (no irritation) to +3 (maximum irritation and redness), was applied to congestion, edema, discharge, and redness of the conjunctiva.[23,26]

IOP measurement

The study adopted a single-dose cross-over design with a 1-week washout period. Twelve rabbits were housed under standard conditions, with a controlled environment (22 ± 0.5°C), alternating light-dark cycles, and access to a standard diet and water.

Glaucoma was induced in rabbit eyes using a steroid induction method. Over 2 weeks, 0.5% dexamethasone eye drops were administered 3 times daily. IOP was measured with a Schiotz tonometer twice weekly at mid-day. After each eye drop, blinking occurred 3 times.

IOP measurements were taken at 0.5, 1, 2, 4, 6, and 24 h post-instillation for both treated and control eyes. The percentage decrease in IOP at each interval was calculated using the formula:

$$\% \text{ decrease in IOP} = \frac{\text{IOP control eye} - \text{IOP treated eye}}{\text{IOP control eye}}$$

Data were analyzed using Kinetica VR 2000 software for parameters such as maximum percentage decrease in IOP (% Dec. IOP max), time for maximum percentage decrease (t_{max}), mean residence time (MRT), and area under the percentage decrease in IOP curve from 0 to 24 h (AUC0–24 h). SPSS software was utilized to assess the significance of differences in these parameters among the selected *in situ* gel formulations.[26-28]

In vivo pharmacokinetics studies

In-vivo pharmacokinetic studies are essential for exploring how a drug or formulation is absorbed, distributed, metabolized, and eliminated within living organisms. These studies yield crucial insights into the drug's behavior, encompassing bioavailability, half-life, clearance (CL), and tissue distribution. Pharmacokinetic parameters, such as maximum plasma concentration (C_{max}), time to reach C_{max}(t_{max}), area under the concentration-time curve (AUC), elimination half-life ($t_{1/2}$), CL, and volume of distribution, are calculated using drug concentration data obtained from the analysis. Software programs or mathematical models are commonly employed for data analysis and parameter estimation.

In this investigation, 16 randomly selected New Zealand rabbits were divided into experimental and control groups. Ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) were administered intramuscularly to induce unconsciousness. The experimental group received 50 µL of a chosen B6 *in situ* gel formulation in the right lower conjunctival sac, while the control group received a timolol eye drop. Local anesthesia was applied with two drops of 1% tetracaine HCl.

Samples of aqueous humor were collected from the anterior chamber at 0.5, 1, 2, 4, 6, and 8 h post-delivery using a 1.0 mL syringe with a 29 G needle. HPLC was employed for material examination. SPSS software was utilized to analyze the outcomes.[27]

Stability studies for optimized in-situ gel

Stability refers to a product's ability to maintain its original properties and characteristics within specified limits throughout its storage and usage period, commonly known as its shelf life. The International Council for Harmonisation has established conditions and procedures for short-term stability studies to predict the physical and chemical stability of drug products.

In the case of ophthalmic preparations, a short-term stability study was conducted for 3 months. Optimized formulations were stored in glass vials under various conditions: 4°C, room temperature (25 ± 2°C) with 60 ± 5% relative humidity (RH), and accelerated temperature (40 ± 2°C) with 65 ± 5% RH. Viscosity, pH, and drug content were assessed at 1, 2, and 3 months to confirm the stability of the optimized formulations.[29]

RESULTS

Clarity and appearance

Various *in situ* gels labeled as B1-B6, containing blueberry extract, were prepared and assessed across different parameters. Visual observations of these formulations revealed diverse appearances, ranging from clear solutions to turbid gels with varying levels of transparency. The notation in Table 2 describes the visual characteristics as follows:

- "Clear": Transparent formulation without visible particles or cloudiness.
- "Turbid": Cloudy or hazy appearance due to suspended particles or aggregates.
- "Transparent (T)": Clear with slight transparency or haziness.
- "Less transparent (L)": Clear, but with reduced transparency compared to a fully transparent solution.

Visual observation is a crucial qualitative assessment offering initial insights into the physical characteristics and stability of the formulations. It helps identify uniform dissolution of the drug and excipients, potential phase separation, or precipitation during gel preparation. Variations in visual

appearance (B1-B6) may stem from differences in blueberry extract concentrations, polymer types, and concentrations. The choice of solvents and mixing order during formulation also influences the final visual appearance of the gels [Table 2].

Table 2: Physicochemical characteristics of ocular *in situ* gel formulations

S. No.	Formulation code	Parameter	
		Clarity	Transparency
1.	B1	Clear	L
2.	B2	Clear	L
3.	B3	Clear	L
4.	B4	Clear	L
5.	B5	Clear	L
6.	B6	Turbid	L

pH

The pH of the formulations (B1-B6) prepared in the study falls within the range of 6.15–6.28. In addition, the pH of the formulations was found to be consistent and unaffected by the presence of the polymers that were investigated during the study. The pH of a formulation is an important parameter that can influence the stability, solubility, and overall performance of the product. The acceptable pH range for ophthalmic formulations, including eye drops, ointments, and *in situ* gels, is typically between 6.0 and 7.4. This pH range is considered optimal for ensuring the compatibility of the formulation with the delicate tissues of the eye and minimizing the risk of eye irritation or discomfort upon application. If the pH of an ophthalmic formulation falls below 6.0, it can become too acidic; this may lead to irritation and damage to the ocular surface. On the other hand, if the pH rises above 7.4, the formulation may become too alkaline, causing a similar risk of irritation and discomfort.

The fact that the pH of the formulations remained within the desired range and was not influenced by the polymers indicates that the formulation process was well controlled, and the selected polymers did not significantly alter the pH of the resulting gels. This is an important finding as it ensures that the formulations are compatible with the ocular environment and are unlikely to cause irritation or discomfort upon application [Table 3].

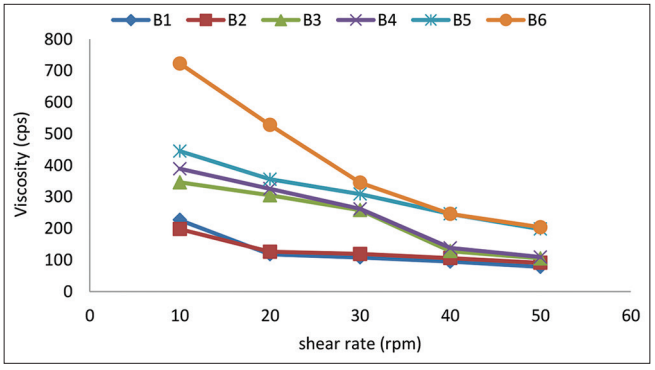
Table 3: pH of the prepared ocular *in situ* gel formulations

S. No	Formulation code	pH
1.	B1	6.15±0.05
2.	B2	6.16±0.06
3.	B3	6.21±0.11
4.	B4	6.56±0.16
5.	B5	6.26±0.08
6.	B6	6.28±0.05

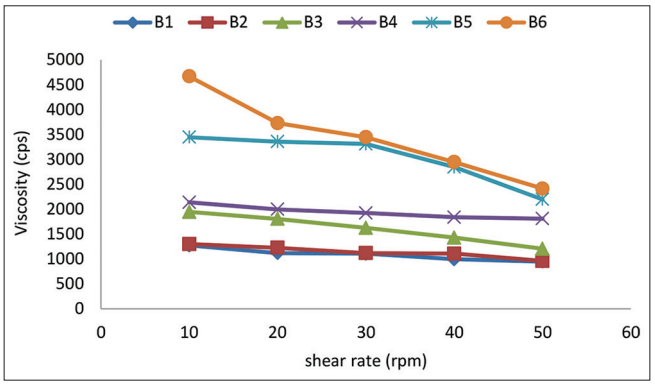
Viscosity

All six formulations exhibited high viscosity under conditions of low shear rates. The viscosity and rheological behavior of the formulations were evaluated both before and after the addition of STF using a Brookfield programmable DV-II + pro model with spindle no. 61 at different shear rates. The graphical representations of viscosity versus shear rate for the *in situ* gels before and after the addition of STF are shown in Graphs 1 and 2.

The rheological behavior of all six formulations showed shear thinning, indicative of pseudo-plastic behavior. Before the addition of STF, the viscosity of formulations B1-B6, the viscosity ranged from 78 to 723 cps before addition of STF and 948 to 4668 cps after addition of STF. These findings indicate that the *in situ* gels demonstrated favorable rheological properties, with their viscosities increasing significantly upon the addition of STF, simulating the physiological environment of the eye. The shear thinning behavior is beneficial for ocular drug delivery, as it ensures easy instillation and spreading of the formulation during application while maintaining the desired drug release characteristics.



Graph 1: Pre-gelation viscosity of ocular *in situ* gel of blueberry extract



Graph 2: Post-gelation viscosity of ocular *in-situ* gel of blueberry extract

Gelling capacity

An ideal *in situ* gelling formulation should possess a well-balanced gelling capacity and viscosity, facilitating effortless administration to the eyes while ensuring efficient gel formation through a swift sol-to-gel transition upon contact at the targeted site. Moreover, it should enhance the drug's residence time at the pre-corneal surface, which is directly influenced by the formulation's viscosity.

Formulation B1, comprising 0.05 g of carbopol 934, showed no gelling ability at the pH of STF (pH 7.4) due to its low viscosity. However, as the concentration of Carbopol 934 was increased, the formulations retained their liquid state at room temperature and the formulated pH. Upon exposure to the pH of STF, the gelling capacity of the formulations improved due to the increased viscosity. Among all the formulations, B5, B6 exhibited exceptional gelling capacity, denoted by "+++". It rapidly formed a robust gel that remained stable for an extended period (more than 6–8 h) [Table 4].

In vitro release studies

The *in vitro* permeation study was conducted using the modified Franz diffusion cell to assess the drug release of all the batches (B1-B6). The results showed that the percentage drug release varied for each formulation over time. Formulations B1 to B3 released 90% of the drug within 4 h and formulation B4 to B6 released 90% of drug in 6 h. The *in vitro* drug release results demonstrate that higher viscosity with stronger gelling ability plays a crucial role in sustaining the drug release from the formulations for an extended period of time. The graphical representation of the percentage cumulative drug release (%) versus time is shown in Graph 3, providing a visual depiction of the drug release patterns of each formulation over the study period.

Ex vivo drug permeation study

The *ex vivo* drug diffusion study was conducted to evaluate the drug permeation from the selected formulation B6 over a period of 8 h. The results indicated that 80.23% of blueberry extract permeated from the formulation, demonstrating a sustained effect. The data, represented in Graph 4, clearly shows that a significantly higher amount of drug permeated from the selected formulations. The *ex vivo* drug diffusion investigation offers significant insights into the drug permeation properties of the chosen formulations, suggesting their potential for prolonged drug release and therapeutic effectiveness. The increased penetration seen in the formulation including blueberry extract indicates its potential efficacy for delivering drugs to the eye. This finding underscores the need of taking into account the physicochemical characteristics of herbal extracts when developing *in situ* gels for targeted therapeutic purposes.

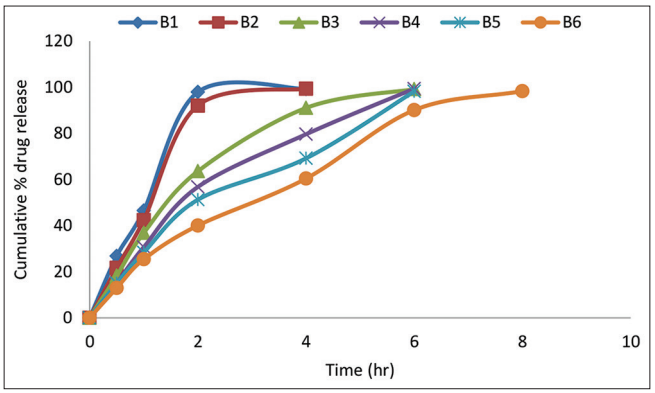
Table 4: *In vitro* gelling capacity of ocular *in situ* gel of blueberry extract

S. No	Formulation code	Gelling capacity
1	B1	-
2	B2	+
3	B3	++
4	B4	++
5	B5	+++
6	B6	+++

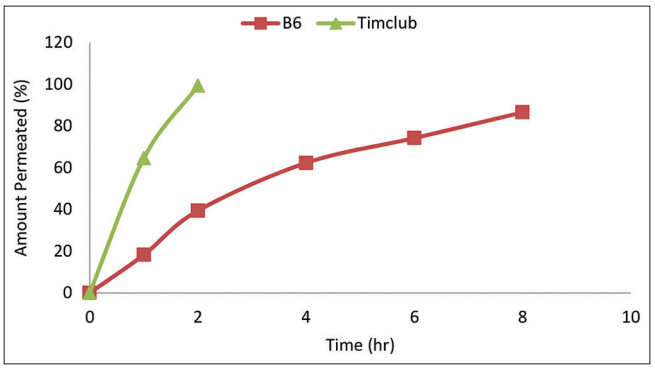
- indicates no gelation, + indicates gelation occurred after few minutes and dissolved rapidly, ++ indicates immediate gelation and remained up to few hours, +++ indicates immediate gelation and remains for extended period

Sterility test

The sterility test was conducted on formulation B6. The test was performed using a Soyabean Casein Digest Medium and the formulation was aseptically transferred into the medium. The mixture was then incubated at a temperature of 35°C for duration of 14 days. The results of the sterility test revealed that there was no evidence of microbial growth in the Soyabean Casein Digest Medium throughout the 14-day incubation period. The absence of visible turbidity or any signs of contamination in the formulation indicated



Graph 3: In vitro release studies of ocular *in situ* gel of blueberry extract



Graph 4: *Ex vivo* corneal permeation study of timolol eye drop, *in situ* gel of B6

that it remained clear and sterile. Based on these findings, the formulation B6 was confirmed to be free from any microbial contamination, meeting the requirements for a sterile ocular product [Table 5]. The successful sterility test ensures the safety and integrity of the formulation, making it suitable for use in ophthalmic applications without the risk of introducing harmful microorganisms to the eye.

In vivo studies on ocular *in situ* gel of blueberry extract (B6)

Ocular irritation test

The ocular irritancy test conducted on the ocular *in situ* gel formulation B6 yielded an overall score of zero, indicating the absence of any signs of ocular irritation, such as redness, increased tear production, or swelling (edema). These results suggest that the excipients used in the formulation are safe for topical application in the eye. The safety of the excipients can be attributed to several factors including biocompatibility and non-irritating properties of herbal extracts and excipients. The excipient used in the formulation has been well established through previous studies for their biocompatibility and non-irritating properties, ensuring that they do not elicit any adverse reactions or harm to the ocular tissues.

The ocular irritancy test demonstrates that the *in situ* gel formulation containing blueberry extract is well tolerated by the eye and does not cause any harmful effects. This is crucial for the development of safe and effective ocular drug delivery systems, ensuring patient compliance and comfort during treatment [Figure 1].

IOP measurement

Graph 5 shows the average percentage reduction in IOP as a function of time for the timolol eye drop, *in situ* gel of blueberry extract (B6) formulations. Glaucoma induction increased the IOP of rabbit eyes from the baseline value of 15.9 ± 2.34 mmHg to 26.6 ± 2.42 mmHg. Maximum IOP reduction of 18.9 ± 2.60 mmHg following ocular administration of timolol eye drop was seen at 2 h; by 5 h, IOP had recovered to pre-administration levels. After 2 h of ocular administration, the *in situ* gel B6 formulation demonstrated the good percentage reduction in IOP, with a value of 29.96 ± 1.47. There was a significant reduction in IOP after using the selected formulations, and this effect was maintained until the end of the trial. The timolol eye drop reduced IOP max by a mean of 36.91 ± 2.68 while it was 29.96 ± 2.88% for B6 formulation. Following a 2-h t_{max}, all the formulations showed a steady reduction in response. In comparison to timolol eye drop, the tested formulation had a considerably greater area under the percentage decrease in IOP response curve (AUC0-24h = 5.104) [Figure 2].

Table 5: Sterility test on ocular *in situ* gel B6

Formulation code	Incubation days							
	1	2	3	4	5	6	7	14
B6	-	-	-	-	-	-	-	-

"-" sign indicates no growth

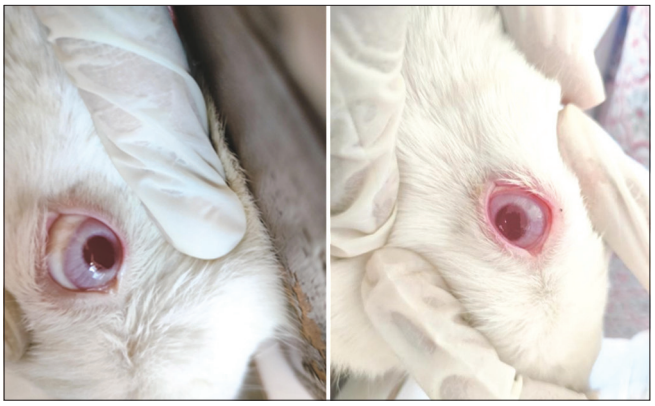
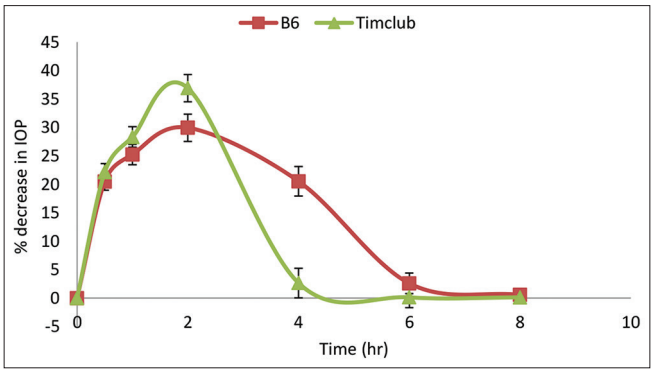


Figure 1: Testing of ocular irritation of control *in situ* gel B6

Figure 2: IOP measurement after application of *in situ* gel formulations containing blueberry extract



Graph 5: Percentage decrease in IOP as a function of time

The MRT was around 1.73 times higher with the *in situ* gel formulations than with the timolol eye drop, suggesting a substantial difference between them. This suggests that the extended pharmacological effect was around 1.73 times greater in the developed formulation.

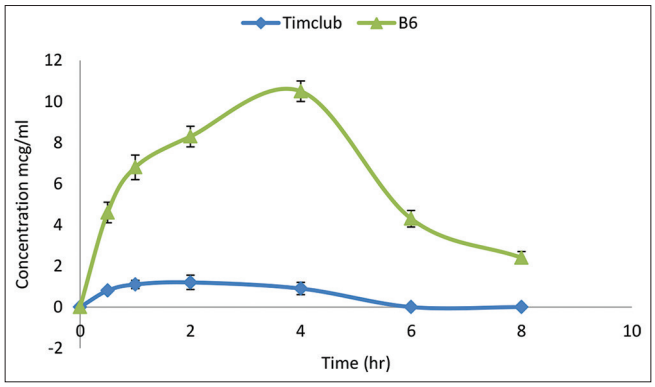
In-vivo pharmacokinetics studies

After the topical administration of the developed *in situ* gel formulations and timolol eye drop, the levels of drugs in the aqueous humor of rabbits were measured over different time intervals, as shown in Graph 6. The pharmacokinetic parameters, including C_{max} (maximum concentration), t_{max} (time to reach C_{max}), AUC, k (elimination rate constant), MRT, and $t_{1/2}$ (half-life), were calculated for both the *in situ* gel formulation and the drug suspension, and the results are presented in Table 6.

The *in situ* gel formulations achieved a C_{max} after 4 h 10 times higher than the C_{max} of the timolol eye drop (0.908 mg/mL) obtained after 2 h. This indicates that the *in situ* gel formulations achieved a significantly higher peak concentration in the aqueous humor compared to the timolol eye drop. The bioavailability in the aqueous humor was also found to be higher compared to the timolol eye drop, as indicated in Table 6. This suggests that the *in situ* gel formulations enhanced the absorption and availability of extracts in the aqueous humor.

Stability studies

During the test duration, the *in situ* gel formulation B6 exhibited good physical form and homogeneity. The stability analysis was performed by subjecting the formulation to various temperatures for a specific period of time. The results of the stability analysis indicated that selected *in situ* gel formulation B6 remained highly stable under these conditions. The stability data, presented in Table 7, provide quantitative information on the performance of the formulation under different temperature conditions. The results of the stability test indicate that B6 formulation is stable and capable of withstanding environmental variations without compromising their quality. This is essential for their intended use as ocular drug delivery systems.



Graph 6: Concentration versus time curve of *in situ* gel formulation (B6) and timclub eye drop in aqueous humor of albino rabbits

Table 6: Pharmacokinetic parameters of *in situ* gel formulations B6 and timclub eye drop

Pharmacokinetic parameters	B6	Timolol eye drop (Timclub)
C_{max} (mcg/mL)	10.5±1.28	0.908±0.048
t_{max}	4 h	2 h
AUC 0–8 (mcg.h/mL)	19.5±2.06	3.04±0.26
K (h-1)	0.684±0.018	0.668±0.215
$t_{1/2}$ (h)	1.25±0.41	0.901±0.164
MRT (h)	3.95±0.26	2.4±0.05

Table 7: Stability data *in situ* gel formulation B6

Storage period	4°C		
	Viscosity (cps)	pH	% drug content
1 month	208±1.24	6.25±0.05	99.18±1.05
2 months	205±1.21	6.19±0.08	98.65±2.18
3 months	204±1.28	6.19±0.12	98.42±2.12
Storage period	**25±2°C and 60±5%RH**		
1 month	208±1.15	6.16±0.09	99.24±0.64
2 months	201±1.32	6.08±0.06	98.39±1.36
3 months	200±1.14	6.04±0.06	97.18±2.42
Storage period	**40±2°C and 65±5%RH**		
1 month	206±1.06	6.18±0.06	99.28±0.72
2 months	200±1.11	6.10±0.05	98.17±1.25
3 months	196±1.45	6.05±0.06	97.15±2.56

CONCLUSION

The formulated ocular *in situ* gels containing blueberry extract represent a promising approach for ocular drug delivery. The comprehensive evaluation of these gels revealed their excellent physical characteristics, including clarity, transparency, and stable pH, their high viscosity, shear-thinning behavior, and exceptional gelling capacity make them suitable for efficient drug delivery to the eye. The *in vitro* and *ex vivo* drug release studies demonstrated sustained drug release, indicating their potential for prolonged therapeutic effects. Importantly, the gels showed no signs of microbial contamination in the sterility test, ensuring their safety for ophthalmic use. Ocular irritation tests confirmed their non-irritating nature, enhancing patient comfort during treatment. The *in situ* gels effectively reduced IOP, suggesting their potential for glaucoma management. Pharmacokinetic analysis revealed significantly improved drug bioavailability in the aqueous humor compared to conventional eye drops. This increased bioavailability, coupled with prolonged drug release, indicates the potential of these *in situ* gel formulations for effective ocular drug delivery and prolonged therapeutic action. Furthermore, stability studies demonstrated the robustness of the selected formulation under various environmental conditions, reinforcing their suitability for practical applications. In conclusion, the ocular *in situ* gels

containing blueberry extract offer a promising platform for ocular drug delivery, with potential benefits for glaucoma treatment and other ophthalmic applications. Further studies and clinical trials are warranted to validate their safety and efficacy in real-world settings.

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ETHICAL APPROVAL

The study was approved by the Institutional Animal Ethics Committee (CPCSEA) of the Pinnacle Biomedical Research Institute (PBRI), Bhopal has provided authorization for the implementation of animal experimentation (Reg. No. 1824/PO/Ere/S/15/CPCSEA).

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