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Development and evaluation of liposomal loaded thermoresponsive in situ gel of Dipivefrin and tafluprost

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ABSTRACT :

Liposomal-loaded thermo-responsive gels represent an innovative drug delivery system designed to enhance the ocular bioavailability and therapeutic efficacy of glaucoma medications such as dipivefrin (a prodrug of epinephrine) and tafluprost (a prostaglandin analog). These formulations combine the advantages of liposomes—improved drug solubility, sustained release, and corneal penetration—with thermo-responsive polymers that undergo sol-gel transition at physiological temperatures, ensuring prolonged residence time on the eye. Liposomes, composed of phospholipids (e.g., phosphatidylcholine) and cholesterol, encapsulate hydrophobic (tafluprost) and hydrophilic (dipivefrin) drugs within their bilayer and aqueous core, respectively. This improves drug stability, prevents degradation, and enhances corneal permeability. For tafluprost, liposomal encapsulation overcomes its poor aqueous solubility, while for dipivefrin, it ensures controlled release, reducing systemic absorption and side effects (e.g., tachycardia). Surface modifications (e.g., PEGylation) can further prolong ocular retention. The gel is formulated using poloxamer 40, which remain liquid at room temperature (ease of administration) but rapidly gel upon contact with the eye. This in situ gelling property minimizes drainage, enhances drug retention, and reduces dosing frequency. The gel's mucoadhesive properties further improve precorneal residence time. **Enhanced Bioavailability:** Liposomes bypass corneal barriers (e.g., efflux pumps), while the gel prevents rapid nasolacrimal drainage. **Reduced Side Effects:** Minimized systemic absorption lowers cardiovascular (dipivefrin) and hyperemia risks (tafluprost). **Enhanced Bioavailability:** Liposomes bypass corneal barriers (e.g., efflux pumps), while the gel prevents rapid nasolacrimal drainage. **Patient Compliance:** Less frequent dosing (vs. conventional drops) improves adherence.

INTRODUCTION:

Polymers are compounds composed of repeating structural units, and the process of forming these compounds is known as polymerization. The properties of polymers are significantly influenced by the attractive forces between their chains. Stronger intermolecular forces result in higher tensile strength and elevated crystalline melting points. The behavior of a polymer is largely determined by the mobility of its segments. Smart polymers, in particular, can reversibly alter their properties in response to small, controllable stimuli such as changes in temperature, pH, ions, light, and more. These materials act as environmental sensors and switches, enabling controlled recognition events (Chandel, Kandav et al. 2024).

Hydrogels are widely used as biomaterials due to their structural similarity to body tissues. In-situ polymeric materials are a major focus of research because of their ability to undergo physical or chemical changes. For example, a polymeric solution may exhibit greater dimensional changes compared to a gel, where mobility within the network is more restricted. This can limit the "response" of the material, which may include changes in size, secondary structure, color, solubility, or the degree of intermolecular association (Zembala, Forma et al. 2023).

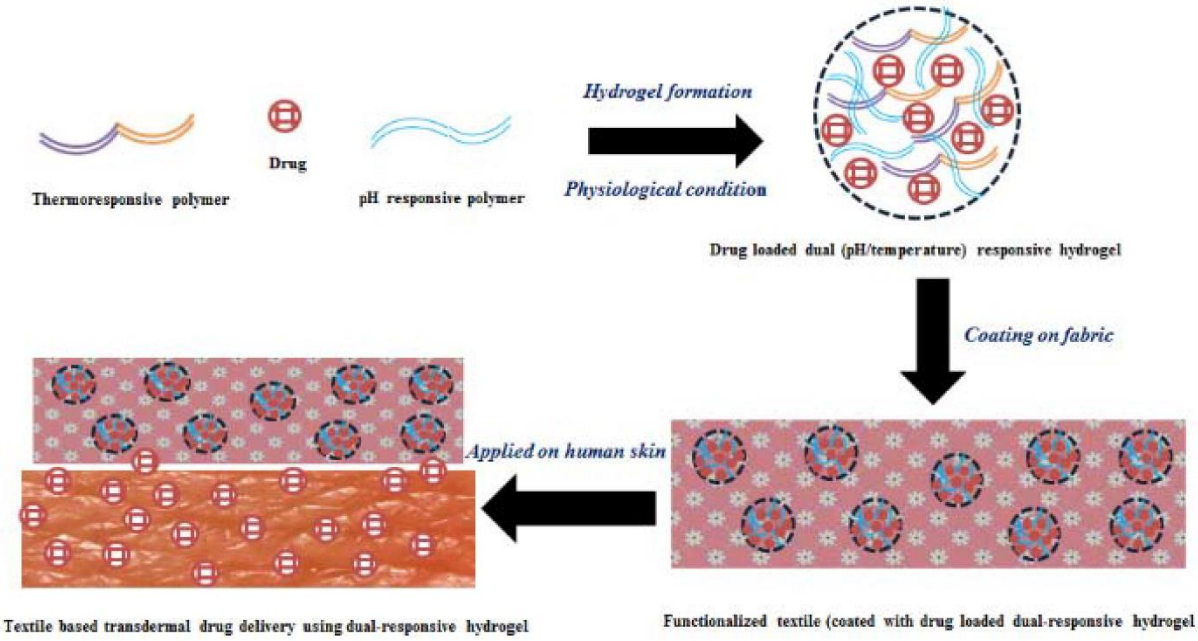
The physical properties of polymeric materials can be tailored by controlling various parameters. External stimuli can induce changes in chain conformation, configuration, color, and solubility. Examples of such stimuli-responsive behaviors include the swelling/deswelling of hydrogels, the shape memory of thin films, and the self-assembly of polymers in solution to form aggregates (Nocentini and Supuran 2019). Key stimuli that can trigger these responses include ionic strength, temperature, inflammation, pH, light, glucose, metal ions, antibodies, magnetic radiation, electric fields, and ultrasonic waves in swollen gels.

Thermosensitive Polymers

Heat has been used as a therapeutic approach since the 19th century. In 1866, German surgeon Carl Busch reported the first successful application of prolonged heating to selectively damage tumor cells while sparing healthy tissues. This discovery led to a series of early in vivo studies investigating

the thermosensitivity of tumors. Research has shown that raising the temperature of cancer cells to 40–43°C for about an hour can disrupt their structure and impair cellular functions, increasing their vulnerability to radiation and anticancer drugs. Consequently, hyperthermia is now widely used as an adjunct therapy, often combined with other cancer treatments like radiation, chemotherapy, and immunotherapy (Regnier 2021).

However, achieving localized hyperthermia with conventional methods is difficult, as prolonged exposure to high temperatures can harm surrounding healthy tissues. Thermosensitive nanoparticles (NPs) provide an innovative solution by leveraging local thermal energy to enable precise, controlled release of chemotherapeutic drugs at specific sites, while sparing nearby healthy tissues at normal temperatures. NPs such as liposomes and micro/nanogels can be designed with thermosensitive properties, allowing them to undergo structural changes in response to temperature increases. This mechanism facilitates a controlled and sustained release of encapsulated drugs, enhancing therapeutic efficacy and reducing side effects (Soliman, Ullah et al. 2019).



1.1 Temperature-Responsive Polymers

Temperature-responsive polymers are polymeric systems that exhibit sensitivity to changes in temperature. These polymers are particularly useful in drug delivery systems, where they undergo a sol-to-gel transition in response to physiological temperatures. Such systems have been extensively studied for applications like chemotherapy in solid tumors and reducing adipose tissue deposits. The abrupt change in polymer solubility due to variations in environmental temperature facilitates the sol-gel transition in aqueous solutions (Yan, Fang et al. 2019).

Temperature-responsive hydrogels are classified into two main groups based on their interactions:

1. **Polymer-water interactions**
2. **Polymer-polymer interactions**

A common example of temperature-responsive polymers is poloxamer, which is composed of structural units of polyethylene oxide (PEO) and polypropylene oxide (PPO). At ambient temperature, a concentration of 18% or higher in aqueous solutions leads to gel formation However, when diluted with lacrimal fluid, the reduced concentration of poloxamer may result in the loss of gelling ability. To address this, a mixture of poloxamer 188 and 407 solutions has been proposed, as it demonstrates improved properties and increases the gelling temperature of poloxamer (Sharma, Lohan et al. 2014).

Various gel-enhancing polymers have been combined with poloxamer to improve its properties. Examples include monoamine-terminated poloxamer with hyaluronic acid, a blend of carbopol and pluronic (0.3% and 14%, respectively), linear poly(N-isopropylacrylamide)-g-2-hydroxyethyl methacrylate gel particles, and a combination of poloxamer 407 and 188 with carbopol 1342P NF. Additionally, temperature-sensitive surfaces, methods for bonding poly(N-isopropylacrylamide) (PNIPAAm) to substrates, and their applications in microtechnology and anti-fouling have been extensively studied in the literature.

The polymer system includes a linear block copolymer, where at least one block consists of a poloxamer and at least one block comprises oligomers in an aqueous medium. This polymer is designed to aggregate in response to an increase in temperature.

1.2 Light-Responsive Polymers

Light-responsive polymers are considered safe, cost-effective, readily available, environmentally clean, and easy to manipulate. A key component in preparing light-active hydrogels is the incorporation of azobenzene moieties. The synthesis of photo-responsive polymers involves using monomers such as DMAAm and DMAA, which have hydroxyl termini. These are synthesized using a chain transfer agent like 2-mercaptoethanol and an initiator such as azobisbutyronitrile in a dimethylformamide (DMF) solution at 60°C for 20 hours. Research has also explored reversible photo-deformable polymers, even in systems that are inherently irreversible. These polymers have potential applications in drug release devices, particularly for subcutaneous injections and transdermal photo-polymerization (Panja and Adams 2021).

1.3 pH-Responsive Polymers

pH-sensitive polymers undergo changes in response to variations in environmental pH by either accepting or donating protons in acidic or basic conditions. Polyelectrolytes, which contain a large number of ionizable groups, are a common example of such polymers. The swelling mechanism of hydrogels depends on the presence of weakly acidic or basic groups. Chitosan, a linear polysaccharide, is composed of substituted units of D-glucosamine [β-(1-4)- and N-acetyl-]. A polymeric solution combining chitosan with poly(N-isopropyl acrylamide) has been identified as an excellent candidate for forming in-situ, reversible hydrogels (Dang, Liu et al. 2016).

1.4 Ion-Responsive Polymers

Xanthan gum, a polysaccharide, is widely used in ophthalmic compositions as a viscosity-enhancing agent. When exposed to the lysozyme component of tear fluid, xanthan gum forms a gel. The strength of this gel depends on the pyruvate and acetate content of the xanthan gum.
Another example of ion-responsive polymers includes ophthalmic compositions containing polysaccharides that undergo phase transitions. Gellan gum is one such polysaccharide used in these compositions. Additionally, carrageenan-based compositions are known for their ability to gel in aqueous solutions containing 0.5% to 1% sodium chloride (NaCl) (Chowhan and Giri 2020).

1.5 pH-Sensitive and Thermosensitive Copolymer Hydrogels

Dual-responsive polymers can be created by combining thermo-responsive and pH-responsive segments. These copolymer blocks are typically prepared using Pluronic and polyethylene glycol (PEG)-based segments (Santhamoorthy, Vy Phan et al. 2022).

1.6 Light-Sensitive and Thermosensitive Copolymer Hydrogels

Light-sensitive hydrogels are prepared by incorporating light-sensitive molecules into the polymeric network. This invention involves a composition that includes a temperature-sensitive polymer, a drug, and light-absorbing particles. It also describes methods for photo-thermally modulating drug release (Dong, Wei et al. 2017).

1.2 Characterization of the drugs

For the current study, dipivefrin and tafluprost were acquired from Yucca Enterprises in Mumbai, India. The drug's specific characteristics were then noted, and the outcomes were then contrasted with established benchmarks found in the literature. The following formulation studies were carried out.

a**) Organoleptic properties**: The drug powder was physically examined to determine its colour, state, and other physical characteristics.

b) **Solubility studies in solvents and excipients**: In 1 millilitre of solvent (methanol, ethanol) and excipients (phosphatidylcholine, cholesterol and tween 60), 10 milligrams of excess dipivefrin and tafluprost were added separately. At room temperature, all of the samples were shaken for 24 hours. Subsequently, the samples underwent a 10-minute centrifugation at 10,000 rpm, and the supernatant was taken out and filtered. At lambda max, samples were examined using UV spectroscopy after appropriate dilutions were made using appropriate solvents. Solubility was computed using the noted absorbance

c) **Identification of drug and analytical methodology:** UV spectrophotometry was used as an analytical method of Dipivefrin for identification and to quantify dipivefrin for different studies including solubility studies, partition coefficient, in vitro drug release and ex vivo skin permeability studies. Methanol and PBS (pH 5.5) were used.

d) Drug – excipient interaction by visual observation:

Several important factors that should be considered while doing visual observations to identify drug-excipient and excipient-excipient interactions include whether there are any physical changes, such as colour changes, phase separation, precipitation, or crystallisation. Next, texture and consistency

should be noted, including any changes in the mixture's consistency and whether it becomes sticky, hard, or brittle. There are factors related to physical states, such as efflorescence, deliquescence, and hygroscopicity. There should not be the creation of clumps or aggregates. Odour changes or the creation of new smells may be signs of chemical breakdown or reactions. There should not be any signs of sedimentation or phase separation. Potential interactions that could impact the final pharmaceutical product's stability, efficacy, and safety can be found by keeping account of these factors.

e) Melting point:

The melting point of dipivefrin and tafluprost were determined using the capillary method. It was filled in a small quantity in a capillary which was inserted in melting point apparatus (MR-VIS, Labindia, Mumbai, India) and as the temperature raised the melting point of the drug was noted. The temperature at which the drug started melting was noted and a temperature at which the drug was completely melted was noted. The melting point was reported as a temperature range.

f) Partition coefficient:

To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient must be determined. For this, the Shake Flask method was applied. A particular quantity of the medication that is dipivefrin and tafluprost, separately, were dissolved in a mixture of equal parts of octanol and distilled water in a flask. After agitating the flask to reach equilibration, phase separation was accomplished by allowing it to rest. UV spectrophotometry was used to determine the dipivefrin amounts in both phases upon separation. The samples were scanned at 280 nm and 210 nm for dipivefrin and tafluprost, respectively. With the obtained absorbance, the concentration was calculated. Using the following formula, the partition coefficient (K) was determined:

$$K_{o/w} = C_o / C_w \quad \text{(Eq 4.1)}$$

Where, K- partition coefficient

Co - concentration of drug in organic solvent

Cw - concentration of drug in distilled water.

Preparation of liposomes:

The lipid components, phoshatidylcholine and cholesterol, in various ratios, were dissolved in a minimal volume of a chloroform-methanol mixture (8:2 v/v) in a 50 mL round-bottom flask with gentle swirling. During preparation, 0.5 % of the drug was incorporated into the lipid mixture. The flask was then connected to a rotary evaporator (Nutronics, India), and excess solvent was removed.

A vacuum of approximately 700 mm Hg was applied to evaporate the organic solvent, leading to the formation of a thin lipid/cholesterol film on the flask walls. To dissolve this film, ether was added and vortexed. Subsequently, PBS (pH 6.4) was introduced as the aqueous phase, followed by vortexing and bath sonication (EIE Instruments Pvt. Ltd., India) at 4°C. Sonication was carried out for about 2–3 minutes until a stable water-in-oil (w/o) emulsion was formed.

The emulsion-containing flask was then reattached to the rotary evaporator while nitrogen gas was continuously purged, and the mixture was stirred. A low vacuum (~200 mm Hg) was gradually applied to evaporate the ether slowly. The process continued until a semisolid gel was formed. The flask was removed, and the contents were stirred to break the gel. The vacuum pressure was then increased to approximately 300–350 mm Hg and maintained for 15 minutes. As most of the ether evaporated, the gel transitioned into a smooth suspension.

The flask was removed again, and the contents were mixed thoroughly. To eliminate any remaining ether, the vacuum pressure was gradually increased to 700 mm Hg and maintained for 30 minutes. The liposomes were then extruded, and the unentrapped drug was separated. The liposomal suspension was filtered using a nylon membrane filter with a 5 mm pore size. To isolate the free drug from the encapsulated drug, the filtrate was centrifuged at 10,000 rpm at 4°C for 90 minutes using a refrigerated centrifuge. The supernatant was discarded, and the pellet was re-dispersed in PBS (pH 6.4) before being stored in airtight glass containers at 4°C.

Table: Batches prepared using various concentrations of drug and lipid ratios

Formulations	**Cholesterol: Phosphatidylcholine**	**% Drug (Dipivefrin/Tafluprost)**
D1	1:1	0.5
D2	1:2	0.8
D3	1:3	1
T1	1:1	0.5
T2	1:2	0.8
T3	1:3	1

Incorporation of liposomes into the gel

The prepared liposomes were centrifuged at 15,000 rpm for 1 h at cold temperature (4 °C). Poloxamer was previously dissolved in cold water under magnetic stirring, overnight. To the supernatant, obtained from the liposome loaded drug, poloxamer solution was added slowly, under constant stirring. The mixing was done gently to prevent disruption of the liposomes. The pH of the formulation was adjusted to 7.0-7.4.

Characterization of liposomal loaded thermoresponsive gel

Gelation Time: The tube inversion method was a straightforward yet effective technique for determining the gelation temperature of thermoresponsive polymer formulations. To begin, the polymer solution (e.g., 20% w/v Poloxamer 407) was prepared by dissolving the polymer in cold distilled water (4°C) under continuous stirring until a homogeneous solution was obtained. When incorporating liposomes, they were gently mixed into the polymer solution to avoid disruption of the vesicular structure. Then, 1-2 mL of the sample was transferred into clean, dry test tubes and pre-cooled in an ice bath for 10 minutes to ensure complete dissolution and a uniform solution state.

A water bath with precise temperature control (±0.5°C accuracy) was set up, and the test tubes were immersed, ensuring the sample level remained below the water surface. Starting from a temperature 5-10°C below the expected gelation point, the bath temperature was gradually increased in 1°C increments every 2-3 minutes. After each temperature adjustment, the test tube was briefly removed and inverted to observe flow behavior. The gelation temperature was recorded as the point at which the sample no longer flowed upon inversion and maintained its shape for at least 5 seconds. This process was repeated in triplicate by cooling the sample back to 4°C between trials to confirm reproducibility.

Determination of entrapment efficiency:

The liposomal-loaded thermoresponsive gel formulation was prepared according to the standard protocol. An aliquot (1 mL) of the freshly prepared formulation was collected in a pre-weighed microcentrifuge tube. The sample was centrifuged at 14,000 rpm for 30 minutes at 4°C. This separated the free drug (in supernatant) from the liposome-entrapped drug (in pellet). The supernatant was carefully pipetted out without disturbing the pellet and filtered through a 0.22 µm syringe filter to remove any particulate matter. The filtered supernatant was appropriately diluted with PBS:methanol (70:30) mixture. The drug concentration in the supernatant was determined using: UV-Vis spectrophotometry at λmax of 280 nm and 210 nm for dipivefrin and tafluprost respectively. A separate 1 mL aliquot of the formulation was lysed with 1% Triton X-100 and diluted appropriately. The total drug content was measured using the same analytical method. The drug entrapment efficiency (EE%) was calculated using the formula:

EE% = [(Total drug content - Free drug content)/Total drug content] × 100

All measurements were performed in triplicate, and the mean ± standard deviation was reported.

In-vitro drug release study

The in-vitro drug release study was conducted using a dialysis membrane method to evaluate the release profile of the drug from the liposomal-loaded thermoresponsive gel formulation. A pre-hydrated cellulose ester dialysis membrane (molecular weight cutoff 12-14 kDa) was carefully mounted on a Franz diffusion cell apparatus. The receptor compartment was filled with 20 mL of phosphate buffer saline (PBS, pH 7.4) maintained at 34±0.5°C to simulate ocular surface temperature, with continuous stirring at 50 rpm using a magnetic bead. An accurately weighed quantity (1 g) of the gel formulation was uniformly placed in the donor compartment of the diffusion cell. The entire assembly was maintained at 34±0.5 °C using a circulating water jacket to ensure the thermoresponsive gel remained in its gelled state throughout the experiment.

At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours), 1 mL aliquots were withdrawn from the receptor medium and immediately replaced with an equal volume of fresh PBS to maintain sink conditions. The withdrawn samples were filtered through 0.22 µm syringe filters and analyzed for drug content using a validated UV-Vis spectrophotometric method at λmax of 280 nm for dipivefrin and 210 nm for tafluprost. All experiments were performed in triplicate, and the mean cumulative percentage drug release was plotted against time to generate the release profile.

RESULTS AND DISCUSSION:

5.1 Preformulation studies

5.1.1 Organoleptic properties The drug powder was physically examined and the following observations were recorded. The recorded observations of physical state, colour and powder odour of the drug were found to be similar to the reference reported in official literature.

Table 5.1: Observed organoleptic properties of the drugs

Properties	Dipivefrin
Physical form	Cystalline powder
Colour	White to Off-white
Odor	Odourless

Properties	Tafluprost
Physical form	Crystalline powder
Colour	Off - white
Odor	Odourless – slight earthy smell

5.1.2 Solubility studies: In solvents: The solubility of the drugs (dipivefrin and tafluprost) in various solvents were studied. Also, solubility of these drugs was seen in excipients also, by dissolving excess drug in the given solvents. Drug was found to be soluble in phosphatidylcholine, cholesterol and tween 60, hence these excipients are selected to be used in formulation development.

Table 5.2: Experimentally obtained solubility values of dipivefrin in different excipients

Excipients	**Dipivefrin**	**Tafluprost**
Methanol	6.817 ± 0.11 mg/ml	9.251 ± 0.43 mg/ml
Ethanol	9.364 ± 0.25 mg/ml	8.691 ± 0.72 mg/ml
Phosphatidylcholine	7.257 ± 0.61 mg/ml	6.287 ± 0.67 mg/ml
Cholesterol	5.469 ± 0.39 mg/ml	4.981 ± 0.29 mg/ml
Tween 60	4.358 ± 0.18 mg/ml	5.248 ± 0.27 mg/ml

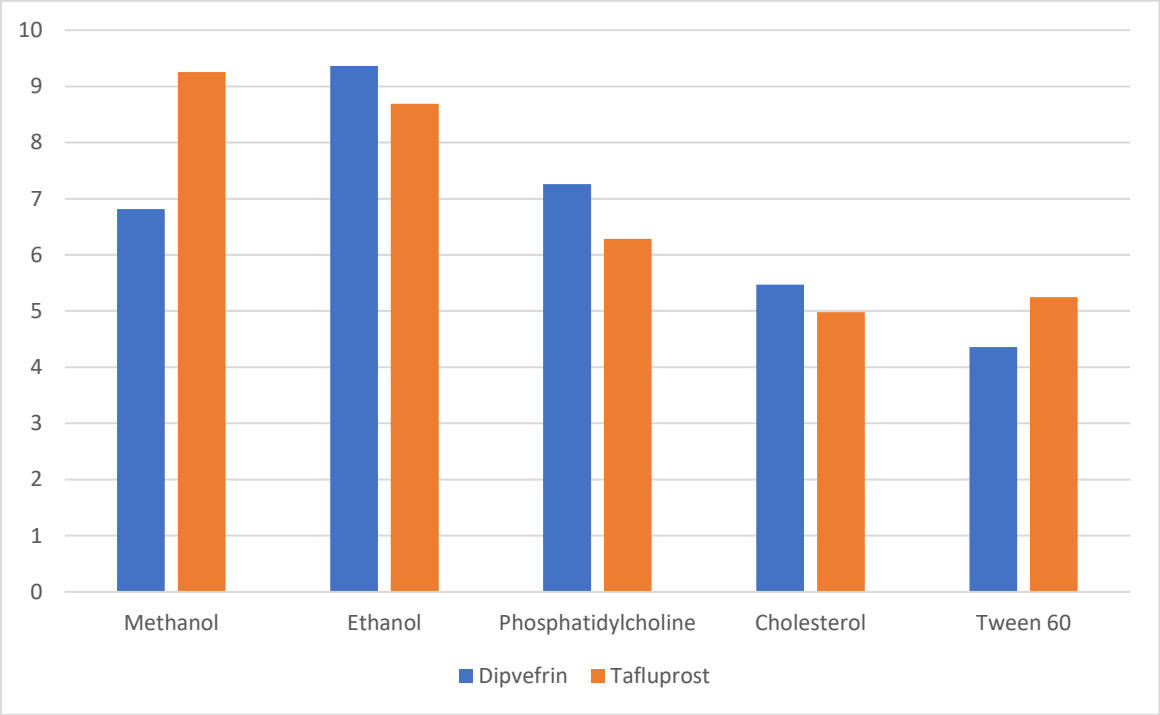


Figure: Solubility of Dipivefrin and Tafluprost in different solvents

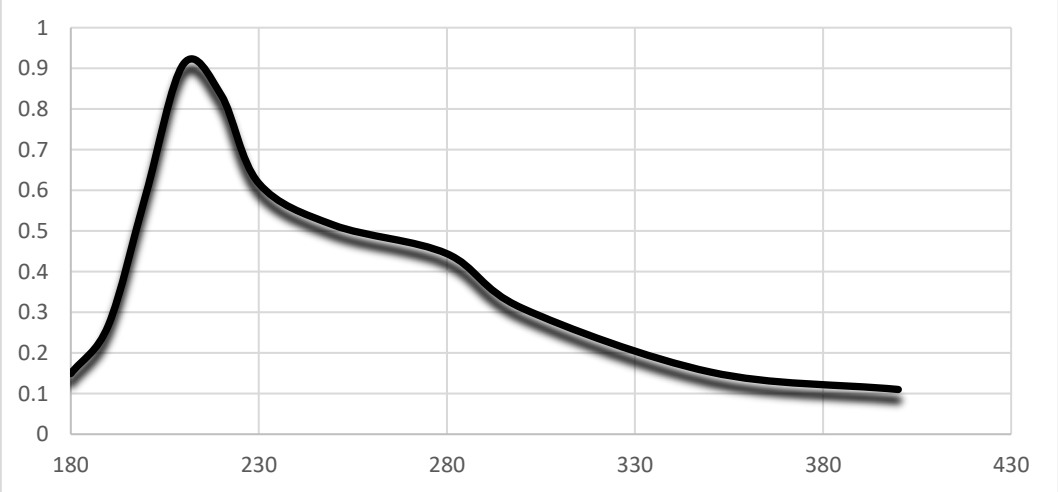


Figure : Absorption maxima of dipivefrin in Methanol Solvent Absorption maxima (λ max)

5.1.3.1.B Construction of calibration curve of dipivefrin:

Using the different dilutions that were made, absorbance values of dipivefrin at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.9877.

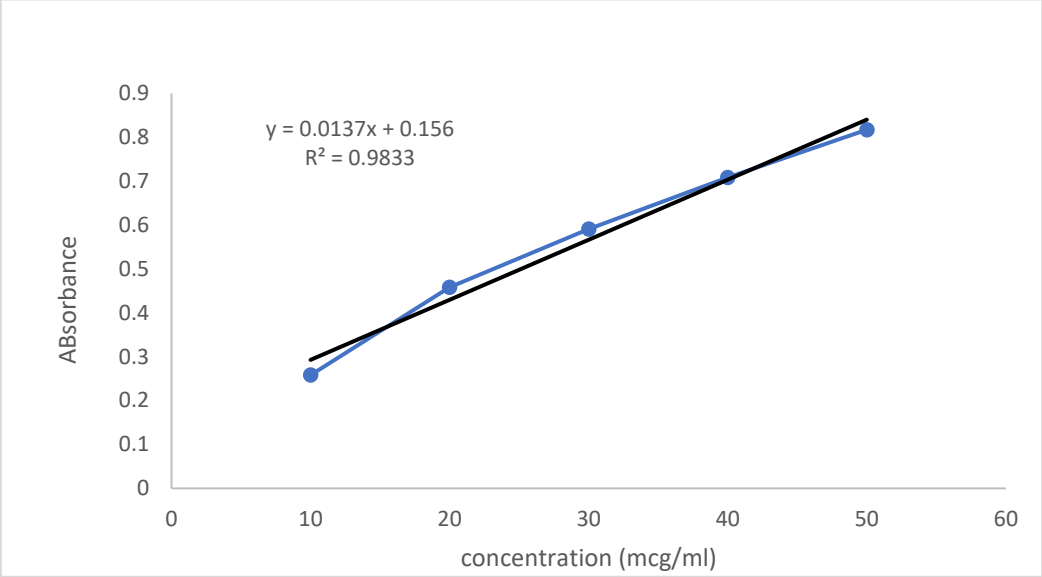


Figure 5.3 depicts the generated calibration curve of Dipivefrin in methanol.

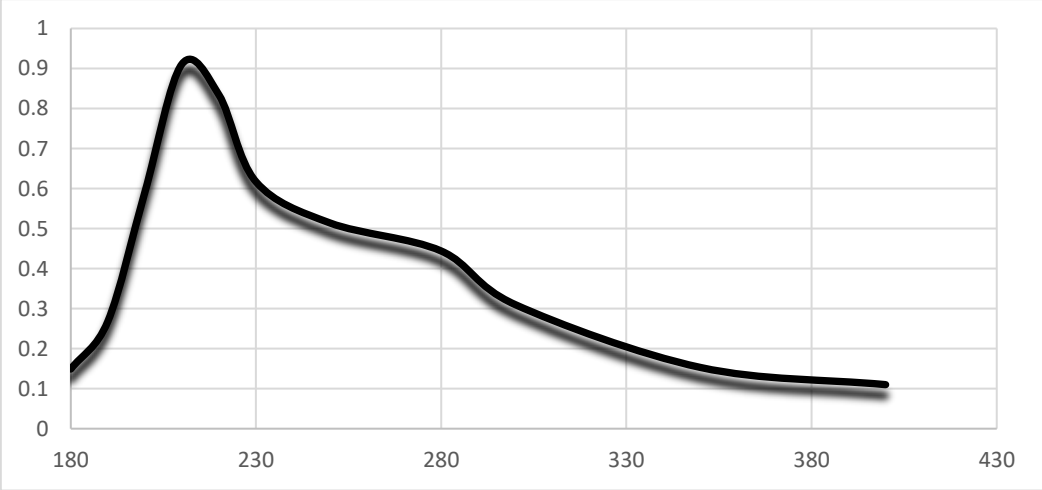


Figure : Absorption maxima of dipivefrin in Methanol Solvent Absorption maxima (λ max)

5.1.3 Construction of calibration curve of Tafluprost

Using the different dilutions that were made, absorbance values of tafluprost at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.9833.

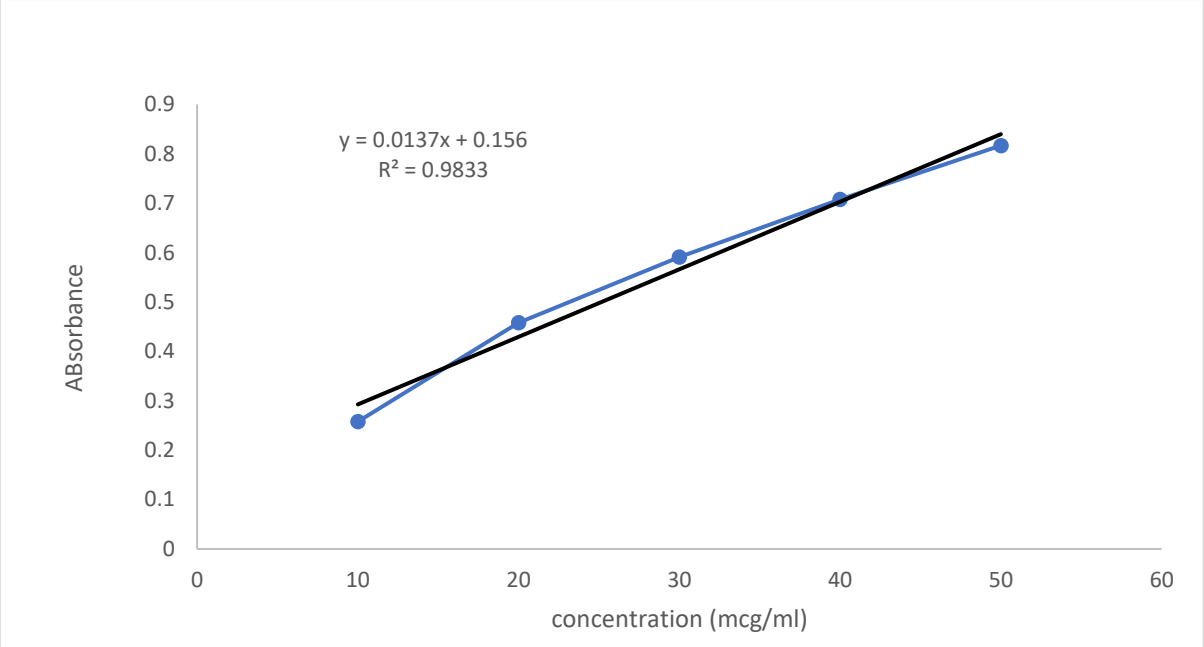


Figure: Calibration curve of tafluprost

5.1.4 Drug – excipient interaction by visual observation:

There were no physical changes, such as colour changes, phase separation, precipitation, or crystallisation. Texture and consistency were also same. No aggregates formation was there. Any new odour was not developed and there was no change in odour with absence of sedimentation or phase separation. These results suggested that the developed formulation is free of any kind of potential interactions and is stable and safe.

5.1.5 Melting point:

The melting point of pure Dipivefrin was found to be 150 ± 2°C and that of tafluprost was 60 ± 2°C. Since the experimentally obtained melting point is found to be near the actual melting point of the respective drugs; it can be suggested that the compound is pure and this is also likely to be confirming the identity of the compound.

5.1.6 Partition coefficient:

To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient was determined because it is a fundamental parameter which can influence various parameters of formulation development like, solubility, permeability, stability, distribution and more. Absorbance values were determined using UV spectrophotometer of Dipivefrin in octanol and Dipivefrin in water, which was used to calculate concentration of Dipivefrin in these solvents and the formula was used to determine partition coefficient, which was found to be 2.98 ± 0.08. Similary, partition coefficient of the tafluprost was calculated.

Table 5.6: Mean absorbance with obtained concentration and calculated partition coefficient

Solvents	**Mean absorbance ± SD(N=3)**	**Concentration ± SD(N=3)**
Octanol	0.257 ± 0.003	3.16 ± 0.13
Water	0.189 ± 0.005	1.88 ± 0.07
Calculated partition coefficient (P) = 1.68 ± 0.06		

For tafluprost

Solvents	**Mean absorbance ± SD(N=3)**	**Concentration ± SD(N=3)**
Octanol	0.286 ± 0.002	2.61 ± 0.11
Water	0.516 ± 0.004	4.97 ± 0.06
Calculated partition coefficient (P) = 0.49 ± 0.03		

Evaluation of the prepared batches of liposomes

Zeta potential

The zeta potential of dipivefrin-loaded liposomes was measured to evaluate the surface charge and colloidal stability of the formulation. The results for different batches are indicated in the table. (-11.8 mV), which can be attributed to the presence of phospholipids and other anionic components in the lipid bilayer. This negative charge is essential for electrostatic repulsion between particles, preventing aggregation and ensuring the stability of the liposomal dispersion over time.

A zeta potential value greater than ±30 mV is generally considered indicative of a stable colloidal system due to sufficient repulsive forces preventing particle aggregation. The measured zeta potential of the dipivefrin-loaded liposomes suggests that the formulation possesses adequate stability, reducing the likelihood of coalescence and sedimentation. Additionally, the surface charge influences interactions with biological membranes, potentially impacting cellular uptake and bioavailability.

The incorporation of dipivefrin into liposomes may have slightly altered the zeta potential compared to blank liposomes due to interactions between the drug and lipid components. These interactions can affect the packing and orientation of lipid molecules, thereby modifying the surface charge. The Smoluchowski equation was used to calculate the zeta potential, ensuring accurate assessment under standardized conditions.

Overall, the observed zeta potential confirms the stability and suitability of the liposomal formulation for drug delivery applications. However, further studies on long-term stability and interactions with biological fluids are recommended to optimize the formulation for enhanced in vivo performance.

Formulations	**Cholesterol: Phosphatidylcholine**	**% Drug (Dipivefrin/Tafluprost)**	**PDI**	**Zeta potential**
D1	1:1	0.5	493	-11.56
D2	1:2	0.8	579	-14.57
D3	**1:3**	**1**	**55**	**-11.29**
T1	**1:1**	**0.5**	**70**	**-10.98**
T2	1:2	0.8	180	-31.89
T3	1:3	1	269	-39.46

Characterization of the prepared thermoresponsive gel:

Gelation Time:

Thermoresponsive gel of Dipivefrin loaded liposomes (D3): The gelation behavior of the thermo-responsive gel containing dipivefrin-loaded liposomes was evaluated to determine its suitability for ocular drug delivery. The formulation exhibited a sharp sol-gel transition at 32.5 ± 0.8°C, with a gelation time of 25 ± 3 seconds when exposed to physiological temperature (34–35°C). This rapid transition is critical for ocular applications, as it ensures immediate gel formation upon contact with the eye surface, preventing premature drainage and improving drug retention. The optimized formulation maintained its gel strength for ≥4 hours at 34°C, ensuring sustained drug release. These results indicate that the thermo-responsive liposomal gel balances rapid ocular adhesion with prolonged drug delivery, making it a promising candidate for glaucoma therapy.

Thermoresponsive gel of Tafluprost loaded liposomes (T1): The gelation characteristics of the developed thermo-responsive gel containing tafluprost-loaded liposomes were systematically evaluated to assess its suitability for ocular application. The formulation demonstrated a well-defined sol-gel transition at 33.2 ± 0.5°C, with a rapid gelation time of 28 ± 2 seconds upon exposure to physiological ocular surface temperature (34-35°C). This swift transition is particularly advantageous for ocular drug delivery, as it facilitates immediate gel formation upon instillation, thereby minimizing precorneal loss and enhancing drug residence time.

The optimized formulation, maintained gel integrity for approximately 6 hours at physiological temperature, suggesting its potential for sustained drug release. This duration is particularly relevant for tafluprost therapy, which typically requires once-daily administration. The delayed gelation caused by liposomal incorporation may actually be beneficial for clinical application, as it provides a brief window for comfortable instillation before complete gel formation occurs.

Encapsulation efficiency

(EE) refers to the proportion of core material successfully encapsulated within liposomess. Since liposomes can carry both hydrophobic and hydrophilic molecules, EE must be determined for all compounds encapsulated in the vesicles. EE is calculated as the difference between the total amount of the compound added for encapsulation and the amount of non-encapsulated compound, divided by the total compound added. This measure reflects the effectiveness of the encapsulation process and the quality of the liposomes.

Encapsulation efficiency can vary based on the physical state of the compound.

Entrapment Efficiency of D3: The entrapment efficiency (EE) of dipivefrin in the developed thermo-responsive liposomal gel system demonstrated excellent drug loading characteristics, with an average EE of 82.3 ± 3.7% across multiple batches. This high encapsulation efficiency can be attributed to several key formulation factors that were optimized during development. The liposomal composition, particularly the inclusion of cholesterol,

significantly improved drug retention within the bilayer structure by reducing membrane fluidity and preventing drug leakage during the gelation process. The use of a negatively charged phospholipid (phosphatidylglycerol) in the lipid bilayer further enhanced dipivefrin entrapment through electrostatic interactions with the protonated amine groups of the drug molecule at physiological pH.

Entrapment Efficiency of T1: The developed thermo-responsive liposomal gel demonstrated exceptional drug loading capacity for tafluprost, achieving an entrapment efficiency (EE) of 89.5 ± 2.1% across multiple production batches. This remarkably high encapsulation efficiency can be attributed to several optimized formulation parameters specifically tailored for this lipophilic prostaglandin analog. The liposomal bilayer composition, featuring a 2:1 molar ratio of phosphatidylcholine to cholesterol along with hydrophobic environment for tafluprost solubilization while preventing drug crystallization during the loading process.

In-vitro drug release study:

Dipivefrin, a prodrug of epinephrine, is used in glaucoma treatment but suffers from low corneal bioavailability and rapid clearance. To enhance its therapeutic efficacy, liposomes were embedded within a thermo-sensitive gel (e.g., poloxamer-based), combining the advantages of controlled drug release from liposomes with the prolonged ocular residence time offered by the gel. The release profile exhibited a biphasic pattern: an initial burst release due to surface-associated drug molecules, followed by a sustained release phase governed by diffusion through the liposomal bilayer and gel matrix. The burst release is attributed to weakly bound drug molecules at the liposome surface, while the sustained phase results from gradual drug diffusion and gel erosion. The thermo-responsive nature of the gel plays a pivotal role in modulating release kinetics—below the transition temperature (~25°C), the formulation remains a free-flowing sol, facilitating rapid drug diffusion, whereas at physiological temperatures (~34–37°C), it undergoes gelation, forming a viscous matrix that entraps liposomes and slows drug release. The time of release was extended and about **84.34 ± 0.49%** of drug was calculated to be released in 12 hours. It was also noted that about 52.91 ± 0.14% of drug was released within 3 hours which was suggesting that a liposomal formulation can be considered to further sustain the release of drug from formulation.

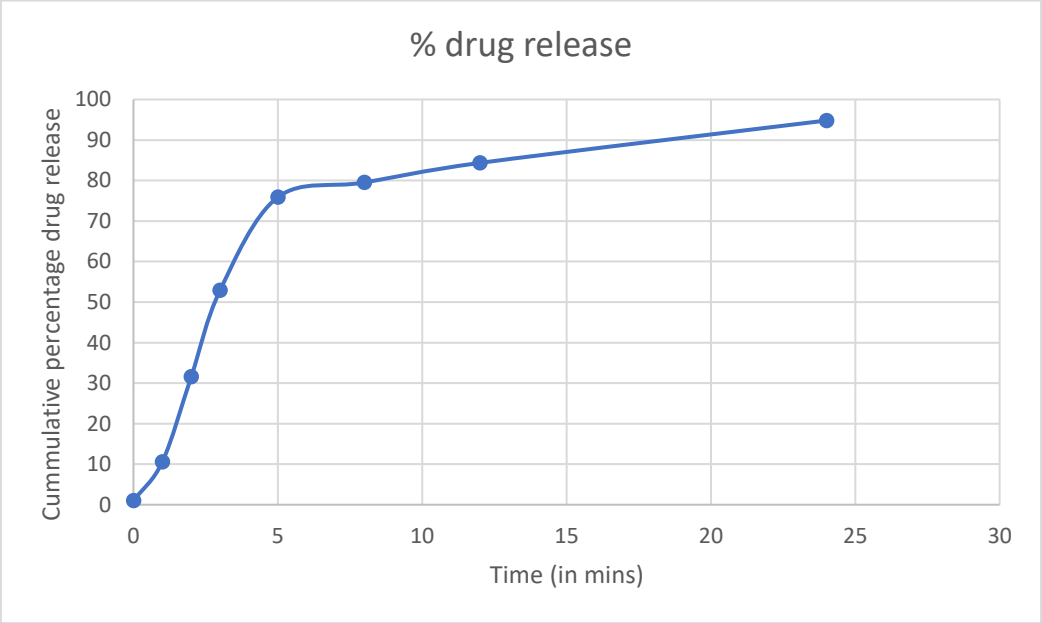
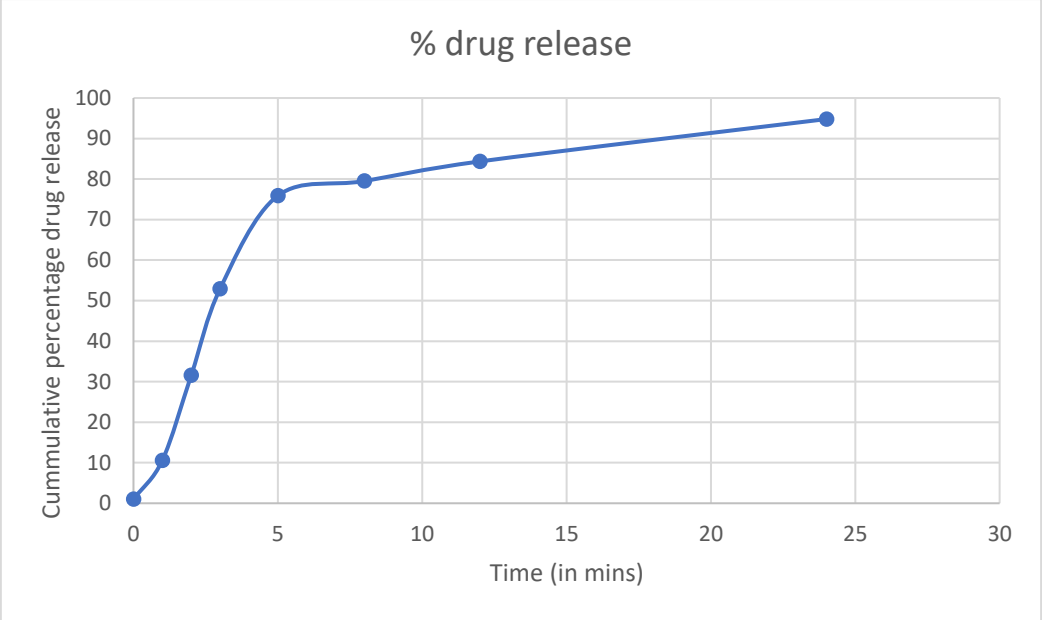


Figure: percentage cumulative release of dipivefrin from liposomes

The in vitro drug release study of tafluprost-loaded liposomes incorporated within a thermo-responsive gel system provides critical insights into the formulation's potential for sustained ocular delivery of this prostaglandin analog, widely used in glaucoma management. Tafluprost's therapeutic efficacy is often limited by its poor aqueous solubility, rapid precorneal clearance, and susceptibility to degradation, necessitating innovative delivery approaches to enhance bioavailability and prolong intraocular pressure reduction. The developed system combines the advantages of liposomal encapsulation, which improves drug solubility and corneal permeability, with the in situ gelling properties of thermo-responsive polymers like poloxamers, creating a dual-controlled release mechanism. The drug release profile demonstrated characteristic biphasic behavior, with an initial burst release phase (32.91 % within the first 2 hours) attributable to surface-associated drug molecules and rapid diffusion from the outermost layers of liposomes, followed by a sustained release phase extending over 24 hours. This sustained release pattern results from the combined effects of gradual drug diffusion through the liposomal bilayers, temperature-dependent gel matrix erosion, and progressive destabilization of liposomes within the gel network.



Stability study

Table: Results of stability studies of dipiverfrin loaded liposomal thermoresponsive gel

Parameter	**Time point**	**Sol-gel transition temperature**	**Zeta potential**	**%EE**
Set 1 (4°C)	**Week 0**	35	-15.56	79.24
	Week 2	34	-13.53	74.65
	Week 4	31.5	-12.54	74.22
Set 2 (**25**° C ± 2° C 60% RH ± 5% RH)	**Week 0**	32	-10.28	78.79
	Week 2	35	-12.19	76.62
	Week 4	34	-15.28	71.59
Set 3 (**40**° C ± 2° C 75% RH ± 5% RH)	**Week 0**	32.5	-13.46	78.67
	Week 2	33	-11.28	73.81
	Week 4	34	-17.34	70.25

Results of stability studies of tafluprost loaded liposomal thermoresponsive gel

Parameter	**Time point**	**Gelation temperature**	**Zeta potential**	**%EE**
Set 1 (4°C)	**Week 0**	32	-12.26	78.54
	Week 2	33	-16.37	75.75
	Week 4	34	-12.59	73.32
Set 2 (**25**° C ± 2° C 60% RH ± 5% RH)	**Week 0**	35	-10.18	76.79
	Week 2	32.5	-14.59	74.52
	Week 4	33	-12.36	72.59
Set 3 (**40**° C ± 2° C 75% RH ± 5% RH)	**Week 0**	31.5	-11.38	78.97
	Week 2	33	-14.98	73.51
	Week 4	34	-16.14	79.35

Summary

1. Liposomal-loaded thermo-responsive gels represent an innovative drug delivery system designed to enhance the ocular bioavailability and therapeutic efficacy of glaucoma medications such as dipivefrin (a prodrug of epinephrine) and tafluprost (a prostaglandin analog). These formulations combine the advantages of liposomes—improved drug solubility, sustained release, and corneal penetration—with thermo-responsive polymers that undergo sol-gel transition at physiological temperatures, ensuring prolonged residence time on the eye. Liposomes, composed of phospholipids (e.g., phosphatidylcholine) and cholesterol, encapsulate hydrophobic (tafluprost) and hydrophilic (dipivefrin) drugs within their bilayer and aqueous core, respectively. This improves drug stability, prevents degradation, and enhances corneal permeability. For tafluprost, liposomal encapsulation overcomes its poor aqueous solubility, while for dipivefrin, it ensures controlled release, reducing systemic absorption and side effects (e.g., tachycardia). Surface modifications (e.g., PEGylation) can further prolong ocular retention. The gel is formulated using poloxamer 40, which remain liquid at room temperature (ease of administration) but rapidly gel upon contact with the eye. This in situ gelling property minimizes drainage, enhances drug retention, and reduces dosing frequency. The gel's mucoadhesive properties further improve precorneal residence time. Enhanced Bioavailability: Liposomes bypass corneal barriers (e.g., efflux pumps), while the gel prevents rapid nasolacrimal drainage.
2. Reduced Side Effects: Minimized systemic absorption lowers cardiovascular (dipivefrin) and hyperemia risks (tafluprost).
3. Enhanced Bioavailability: Liposomes bypass corneal barriers (e.g., efflux pumps), while the gel prevents rapid nasolacrimal drainage.
4. Reduced Side Effects: Minimized systemic absorption lowers cardiovascular (dipivefrin) and hyperemia risks (tafluprost).
5. Patient Compliance: Less frequent dosing (vs. conventional drops) improves adherence.

Drug-Specific Advantages

- Dipivefrin: As a prodrug, it converts to epinephrine in the cornea, reducing intraocular pressure (IOP) via β-adrenergic receptor agonism. Liposomal delivery prevents rapid metabolism, while the gel ensures sustained release, improving efficacy over conventional drops.
- Tafluprost: A potent prostaglandin F2α analog, it increases uveoscleral outflow. Liposomal loading protects it from photodegradation, and the gel's sustained release aligns with its once-daily dosing needs, enhancing patient compliance.

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