CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.20719967

Available online at: http://www.iajps.com Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF THERMOREVERSIBLE OCULAR IN SITU HYDROGEL OF OLOPATADINE HYDROCHLORIDE FOR SUSTAINED OPHTHALMIC DRUG DELIVERY

Sana Dildar Mujawar*, Dr. Narhari Amol Yedake, Dr. Ravi Uttamrao Kurhade, Mr. Nishinandan Manikrao Shinde
MDA School of Pharmacy, Kolpa, Latur, Maharashtra 413531

Abstract:
The present study aimed to develop and evaluate a thermoreversible ocular in situ hydrogel of Olopatadine Hydrochloride for sustained ophthalmic drug delivery. The formulation was designed to overcome the limitations of conventional eye drops, including poor ocular bioavailability, rapid precorneal elimination, and frequent dosing requirements. Preformulation studies confirmed the purity and compatibility of the drug with selected excipients. Thermoreversible in situ hydrogels were prepared using Poloxamer 407, Poloxamer 188, HPMC K4M, and Carbopol 940 by the cold method. The prepared formulations were evaluated for physicochemical parameters such as appearance, pH, drug content, viscosity, gelling capacity, isotonicity, sterility, and in vitro drug release. All formulations exhibited acceptable characteristics for ophthalmic application. Among them, formulation F9 demonstrated optimal performance with suitable pH, satisfactory drug content, rapid gelation, appropriate viscosity, and prolonged drug release. The optimized formulation showed sustained release behavior and followed the Korsmeyer–Peppas kinetic model with a non-Fickian diffusion mechanism. Stability studies indicated good formulation stability under accelerated storage conditions. The developed thermoreversible ocular in situ hydrogel of Olopatadine Hydrochloride offers a promising approach for sustained ophthalmic drug delivery by enhancing ocular residence time, improving bioavailability, reducing dosing frequency, and increasing patient compliance.

KEYWORDS: *Olopatadine Hydrochloride, Thermoreversible Hydrogel, Ocular In Situ Gel, Sustained Drug Delivery, Ophthalmic Formulation, Ocular Bioavailability.*

Corresponding author:
Sana Dildar Mujawar,
M. Pharm Scholar,
MDA School of Pharmacy, Kolpa, Latur, Maharashtra 413531
Email: mujawarsana05@gmail.com

QR CODE

Please cite this article in press Sana Dildar Mujawar et al., Formulation Development And Evaluation Of Thermoreversible Ocular In Situ Hydrogel Of Olopatadine Hydrochloride For Sustained Ophthalmic Drug Delivery, Indo Am. J. P. Sci, 2026; 13(06).

INTRODUCTION:

The eye is a highly specialized and sensitive organ, and the effective delivery of therapeutic agents to ocular tissues remains a significant challenge in pharmaceutical research. Conventional ophthalmic formulations such as eye drops are the most commonly used dosage forms for the treatment of ocular disorders; however, their therapeutic effectiveness is often limited due to rapid precorneal elimination, tear turnover, nasolacrimal drainage, and poor corneal permeability.[1-4] As a result, only a small fraction of the administered drug reaches the target site, leading to low ocular bioavailability, frequent dosing requirements, and reduced patient compliance. To overcome these limitations, novel ocular drug delivery systems have been developed to enhance drug residence time and improve therapeutic efficacy. Among these approaches, in situ gelling systems have gained considerable attention due to their ability to undergo a phase transition from a liquid state to a gel state upon exposure to physiological conditions. Thermoreversible in situ hydrogels are particularly advantageous because they remain as free-flowing liquids during administration and transform into gels at ocular temperature, thereby prolonging contact time with the ocular surface and reducing drug loss.[5-9]

Olopatadine Hydrochloride is a selective histamine H1-receptor antagonist and mast cell stabilizer widely used in the treatment of allergic conjunctivitis and other ocular allergic conditions. Although conventional Olopatadine eye drops provide rapid relief from allergic symptoms, their short precorneal residence time necessitates repeated administration to maintain therapeutic drug levels.[10-12] Therefore, the development of a sustained-release ocular delivery system for Olopatadine Hydrochloride is desirable to improve drug retention, enhance bioavailability, and reduce dosing frequency. Thermosensitive polymers such as Poloxamer 407 and Poloxamer 188 are extensively employed in the formulation of ocular in situ gels because of their temperature-dependent gelation properties. The incorporation of viscosity-enhancing and mucoadhesive polymers such as Hydroxy Propyl Methyl Cellulose (HPMC K4M) and Carbopol 940 further improves gel strength, ocular retention, and controlled drug release characteristics. The combination of these polymers can provide a stable and effective drug delivery platform for sustained ophthalmic therapy. The present study was undertaken to formulate and evaluate a thermoreversible ocular in situ hydrogel of Olopatadine Hydrochloride using a combination of thermosensitive and mucoadhesive polymers.[13-15] The developed formulations were evaluated for their physicochemical characteristics, gelling behavior, drug release profile, sterility, stability, and release kinetics. The objective was to develop a sustained ophthalmic drug delivery system capable of improving ocular residence time, enhancing therapeutic efficacy, reducing dosing frequency, and improving patient compliance.[16-18]

MATERIALS AND METHODS:

Materials:

Olopatadine Hydrochloride was obtained from Yarrow Chem Products, Mumbai, India, and used as the active pharmaceutical ingredient. Poloxamer 407 and Poloxamer 188 were procured from BASF India Ltd., Mumbai, and employed as thermosensitive polymers. Hydroxy Propyl Methyl Cellulose (HPMC K4M) and Carbopol 940 were obtained from S.D. Fine Chem. Ltd., Mumbai, and used as viscosity-enhancing and mucoadhesive agents, respectively. Benzalkonium chloride was used as a preservative, while sodium chloride served as a tonicity-adjusting agent. Disodium hydrogen phosphate, citric acid, sodium hydroxide, and hydrochloric acid were utilized for buffering and pH adjustment. Distilled water was used as the vehicle for formulation preparation. Simulated tear fluid, prepared in the laboratory, was employed as the dissolution medium for in vitro drug release studies.

Methodology:

Preformulation Studies

Preformulation studies of Olopatadine Hydrochloride were carried out to evaluate its physicochemical properties and compatibility with selected excipients. The drug was characterized for organoleptic properties, solubility in different solvents, and melting point determination. Drug–excipient compatibility was assessed by Fourier Transform Infrared Spectroscopy (FTIR) using the KBr pellet method over a scanning range of 4000–400 cm^{-1}. The obtained spectra of the pure drug and physical mixtures with polymers were analyzed for possible interactions.[19-22]

Determination of λ-max and Calibration Curve

The λ-max of Olopatadine Hydrochloride was determined using UV–Visible spectrophotometry. A standard stock solution was prepared in Simulated Tear Fluid (STF, pH 7.4) and scanned in the wavelength range of 200–400 nm to identify the wavelength of maximum absorbance. A calibration curve was then constructed by preparing serial dilutions in the concentration range of 1–10 µg/ml and measuring their absorbance at the selected λ-max. The calibration plot of absorbance versus concentration was used for quantitative estimation of drug content and in vitro drug release studies.[23-25]

Preparation of Thermoreversible Ocular In Situ Hydrogel

Thermoreversible ocular in situ hydrogel formulations of Olopatadine Hydrochloride were prepared by the cold method using Poloxamer 407 and Poloxamer 188 as thermosensitive polymers. The required quantities of polymers were gradually dispersed in cold distilled water (4°C) under continuous magnetic stirring and refrigerated overnight to ensure complete hydration and formation of a clear solution. HPMC K4M and Carbopol 940 were subsequently incorporated into the polymeric solution and allowed to hydrate completely with continuous stirring.[26-30]

Olopatadine Hydrochloride was dissolved separately in distilled water, followed by the addition of benzalkonium chloride as a preservative and sodium chloride as a tonicity-adjusting agent. The drug solution was then added slowly to the polymeric mixture with constant stirring. The pH of the formulation was adjusted to 7.4 using a suitable buffer system, and the final volume was made up with distilled water. The prepared formulations (F1–F10) were stored under refrigerated conditions until further evaluation. The formulations remained as free-flowing liquids at room temperature and transformed into gels at ocular physiological temperature, thereby enhancing ocular residence time and providing sustained drug release.[31-35]

Table 1: Formulation Composition of Thermoreversible Ocular In Situ Hydrogel of Olopatadine Hydrochloride

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Olopatadine Hydrochloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Poloxamer 407	16	17	18	19	20	18	19	20	21	22
Poloxamer 188	4	4	4	4	4	5	5	5	5	5
HPMC K4M	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.4
Carbopol 940	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.3
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Buffer Solution (pH 7.4)	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml

Evaluation of Thermoreversible Ocular In Situ Hydrogel[36-57]

Physical Appearance

The prepared formulations were visually evaluated for color, clarity, homogeneity, consistency, and the presence of particulate matter under normal daylight conditions. The formulations were examined to ensure transparency, smoothness, and suitability for ophthalmic administration.

Determination of pH

The pH of each formulation was measured using a calibrated digital pH meter. The measurements were performed to ensure compatibility with the physiological pH of tear fluid and to minimize ocular irritation.

Drug Content Determination

Drug content was determined by UV–Visible spectrophotometry. Appropriately diluted samples of the formulations were analyzed at the predetermined λ-max, and the drug content was calculated using the standard calibration curve of Olopatadine Hydrochloride.

***In Vitro* Gelling Capacity**

The gelling capacity of the formulations was evaluated by adding a drop of formulation into Simulated Tear Fluid (STF, pH 7.4) maintained at physiological temperature. Gel formation, gel strength, and the duration of gel integrity were visually observed and recorded.

Determination of Gelling Time

Gelling time was determined by measuring the time required for the formulation to undergo sol-to-gel

transition after contact with STF at physiological temperature. Rapid gelation was considered desirable for effective ocular retention.

Determination of Viscosity
The viscosity of the formulations was measured using a Brookfield viscometer at room temperature and physiological temperature. The rheological behavior of the formulations was evaluated to ensure ease of installation and prolonged ocular residence.

Isotonicity Testing
Isotonicity was assessed microscopically by observing the morphology of red blood cells after exposure to the formulations. The absence of cellular swelling or shrinkage indicated isotonic compatibility with ocular tissues.

Spreadability Study
Spreadability was evaluated using the glass slide method by measuring the ease with which the formulation spread between two glass slides. Adequate spreadability is essential for uniform ocular distribution and enhanced therapeutic performance.

***In Vitro* Drug Release Study**
In vitro drug release studies were carried out using a Franz diffusion cell equipped with a dialysis membrane. Simulated Tear Fluid (pH 7.4) maintained at 37 ± 0.5°C was used as the receptor medium. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically to determine cumulative drug release.

Sterility Testing
Sterility testing was performed according to Indian Pharmacopoeia guidelines using the direct inoculation method. Sterilized formulations were inoculated into Soybean-Casein Digest Medium and incubated at 37°C for seven days. The absence of microbial growth confirmed the sterility of the formulations.

Kinetic Drug Release Study
The in vitro drug release data of the thermoreversible ocular in situ hydrogel formulations were analyzed using various kinetic models, including Zero-order, First-order, Higuchi, Hixson-Crowell, and Korsmeyer–Peppas models. The release data were fitted into these mathematical models to determine the drug release pattern and elucidate the underlying release mechanism. The model exhibiting the highest correlation coefficient (R^2) value was considered the best fit for describing the release kinetics of the formulation.

Zero-Order Kinetics
The zero-order model was applied to evaluate whether drug release occurred at a constant rate independent of drug concentration. A plot of cumulative percentage drug released versus time was constructed to assess the release behavior.

First-Order Kinetics
The first-order model was used to determine whether the drug release rate was dependent on the concentration of drug remaining in the formulation. A plot of log cumulative percentage drug remaining versus time was analyzed.

Higuchi Model
The Higuchi model was employed to investigate diffusion-controlled drug release from the polymeric matrix system. A plot of cumulative percentage drug released versus the square root of time was used to evaluate the diffusion mechanism.

Hixson-Crowell Model
The Hixson-Crowell model was applied to assess the influence of changes in surface area and particle dimensions on drug release. The model was used to evaluate erosion and dissolution characteristics of the formulation.

Korsmeyer–Peppas Model
The Korsmeyer–Peppas model was utilized to determine the mechanism of drug release from the polymeric hydrogel system. The diffusion exponent (n) obtained from the model was used to identify whether the release followed Fickian diffusion, non-Fickian transport, or erosion-controlled release.

Stability Study
Stability studies of the optimized formulation were conducted according to ICH guidelines under accelerated storage conditions (40 ± 2°C and 75 ± 5% RH) for a period of two months. The formulation was stored in amber-colored, tightly closed glass containers and evaluated at predetermined intervals for physical appearance, pH, drug content, viscosity, gelling capacity, and *in vitro* drug release. The study was performed to assess the stability and integrity of the formulation during storage.

Comparison with Marketed Product
The optimized thermoreversible ocular in situ hydrogel formulation was compared with a marketed ophthalmic preparation to evaluate its drug release characteristics and overall performance. Comparative in vitro diffusion studies were carried out using a Franz diffusion cell under identical experimental conditions. The cumulative percentage drug release and sustained release behavior of the optimized formulation were

compared with those of the marketed product to assess its potential for improved ocular retention and prolonged drug delivery.

RESULTS AND DISCUSSION:

Preformulation Studies of Olopatadine Hydrochloride

Preformulation studies were performed to evaluate the physicochemical characteristics and compatibility of Olopatadine Hydrochloride prior to formulation development. The results obtained from organoleptic evaluation, solubility analysis, and melting point determination confirmed the identity, purity, and suitability of the drug for the development of thermoreversible ocular in situ hydrogel formulations.

Organoleptic Evaluation

The organoleptic evaluation revealed that Olopatadine Hydrochloride was a white to off-white, odorless crystalline powder with a fine and free-flowing nature. These observations were consistent with the reported standard specifications, indicating the purity and acceptable quality of the drug sample.

Solubility Analysis

The solubility study demonstrated that Olopatadine Hydrochloride was freely soluble in distilled water, phosphate buffer pH 7.4, and simulated tear fluid, while exhibiting good solubility in methanol and slight solubility in ethanol. The favorable aqueous solubility profile supports its suitability for ophthalmic formulation development and facilitated the preparation of the in situ hydrogel system.

Melting Point Determination

The melting point of Olopatadine Hydrochloride was found to be in the range of 250–253°C, which is consistent with the reported literature values. The sharp melting range indicated the absence of significant impurities and confirmed the purity and thermal stability of the drug.

Table 2: Preformulation Study Results

Parameter	Observation/Result	Interpretation
Color	White to off-white	Complies with standard
Appearance	Crystalline powder	Pure drug sample
Odor	Odorless	No degradation detected
Nature	Fine, free-flowing powder	Suitable for formulation
Solubility in Water	Freely soluble	Suitable for ophthalmic delivery
Solubility in STF	Freely soluble	Compatible with ocular environment
Melting Point	250–253°C	Confirms purity and stability

FTIR Study of Drug and Drug–Polymer Compatibility

FTIR spectroscopy was performed to identify the characteristic functional groups of Olopatadine Hydrochloride and to evaluate its compatibility with the selected formulation excipients, namely Poloxamer 407, Poloxamer 188, HPMC K4M, and Carbopol 940. The spectra of the pure drug and drug–polymer physical mixtures were recorded in the range of 4000–400 cm^{-1} using the KBr pellet method. The characteristic peaks corresponding to hydroxyl, carbonyl, ether, and tertiary amine functional groups confirmed the identity and purity of Olopatadine Hydrochloride. Comparative analysis of the spectra revealed no significant shift, disappearance, or formation of additional peaks, indicating the absence of chemical interactions between the drug and the selected polymers.

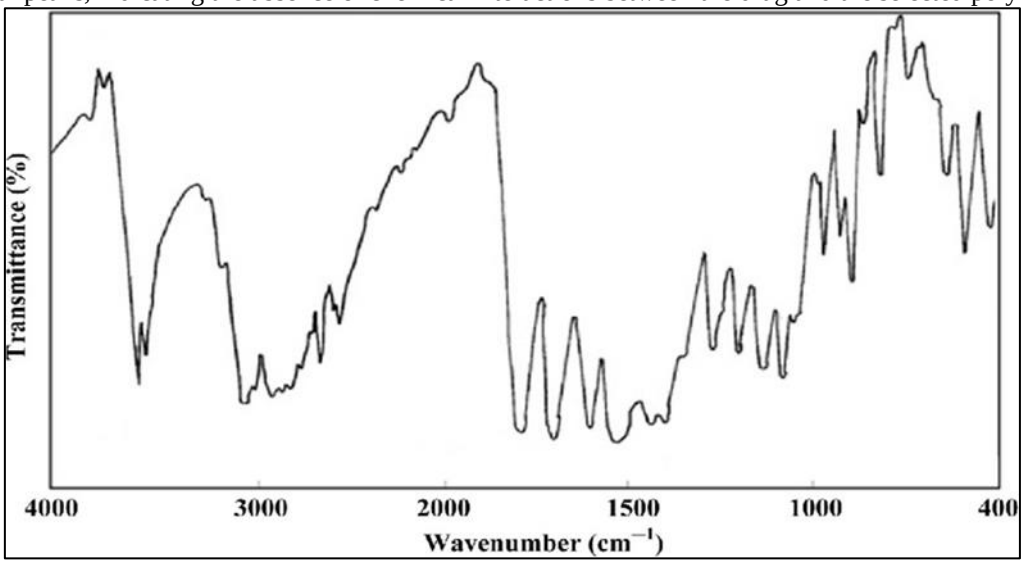

Figure 1: FTIR Spectrum of Pure Olopatadine Hydrochloride

The FTIR compatibility study demonstrated that the characteristic functional groups of Olopatadine Hydrochloride remained unchanged in the presence of Poloxamer 407, Poloxamer 188, HPMC K4M, and Carbopol 940. No significant peak shifting, disappearance, or formation of new peaks was observed in any of the drug–polymer mixtures, confirming the absence of chemical incompatibility. These findings established the compatibility of the selected excipients with Olopatadine Hydrochloride and supported their use in the development of thermoreversible ocular in situ hydrogel formulations.

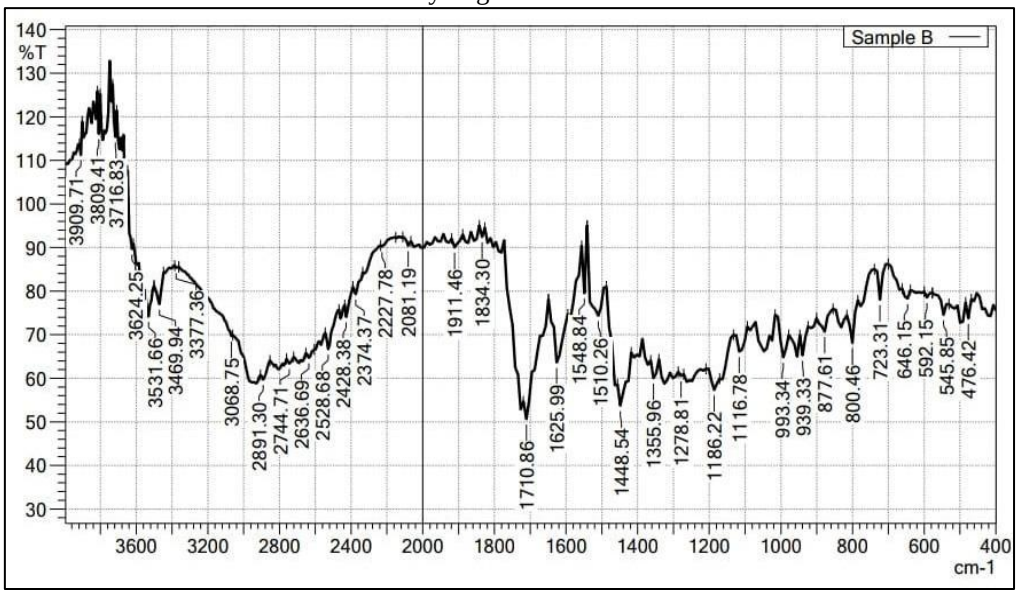

Figure 2: FTIR Spectrum of physical mixture

Determination of Wavelength (λ-max) of Olopatadine Hydrochloride

The UV absorption spectrum of Olopatadine Hydrochloride in Simulated Tear Fluid (STF, pH 7.4) was recorded in the wavelength range of 200–400 nm. The drug exhibited a distinct absorption maximum (λ-max) at 299 nm, which was selected as the analytical wavelength for subsequent quantitative estimations. The sharp and well-defined absorption peak confirmed the suitability of STF as the analytical medium and demonstrated the stability of the drug under experimental conditions.

Table 3: Optical Characteristics of Olopatadine Hydrochloride

Sr. No.	Parameter	Observation
1	λ-max	299 nm
2	Analytical Medium	Simulated Tear Fluid (pH 7.4)
3	Wavelength Range Scanned	200–400 nm
4	Absorption Peak	Sharp and well-defined

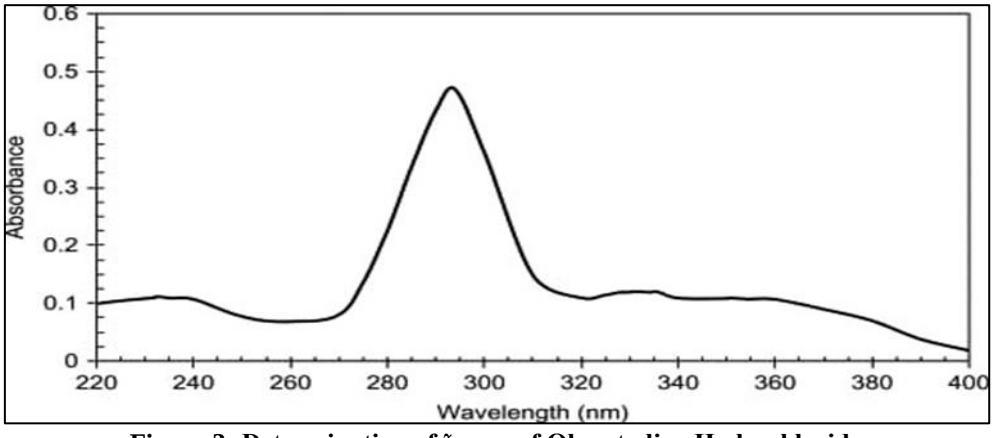

Figure 3: Determination of ƛ max of Olopatadine Hydrochloride

Calibration Curve of Olopatadine Hydrochloride

The calibration curve of Olopatadine Hydrochloride was constructed in STF (pH 7.4) at 299 nm using concentrations ranging from 1–10 µg/ml. The absorbance increased proportionally with concentration, indicating excellent linearity and compliance with Beer–Lambert's law. The developed UV spectrophotometric method was found to be accurate, reliable, and suitable for drug content determination and in vitro drug release studies.

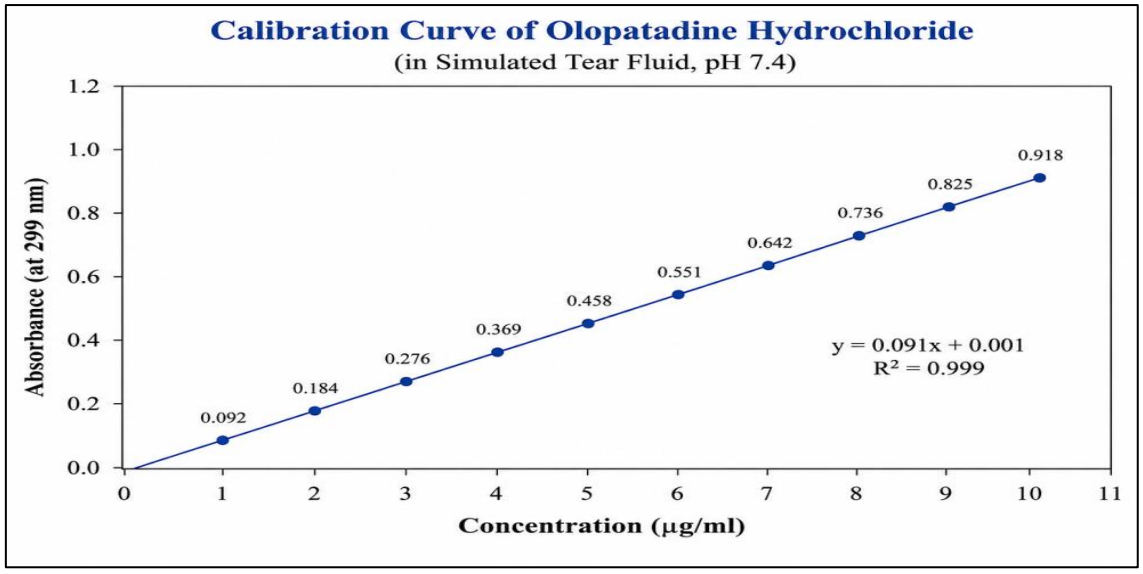

Figure 4: Calibration curve of Olopatadine Hydrochloride

The high correlation coefficient (R^2 = 0.999) demonstrated excellent linearity between concentration and absorbance within the selected range. These findings confirmed that the developed UV spectrophotometric method was suitable for quantitative estimation of Olopatadine Hydrochloride in formulation and release studies.

Preparation of Thermoreversible Ocular In Situ Hydrogel

Thermoreversible ocular in situ hydrogel formulations of Olopatadine Hydrochloride were successfully prepared by the cold method using Poloxamer 407 and Poloxamer 188 as thermosensitive polymers, HPMC K4M as a viscosity-enhancing agent, and Carbopol 940 as a mucoadhesive polymer. A total of ten formulations (F1–F10) were developed by varying polymer concentrations to optimize gelation characteristics, viscosity, ocular retention, and drug release behavior.

All formulations were clear, transparent, homogeneous, and free from visible particulate matter or phase separation. The formulations remained as free-flowing liquids at room temperature and rapidly transformed into gels at physiological ocular temperature, confirming successful thermoreversible behavior. The concentration of polymers significantly influenced viscosity, gel strength, gelling capacity, and drug release characteristics. Increasing the concentration of Poloxamer 407 and Carbopol 940 enhanced gel strength and ocular retention, while HPMC K4M contributed to increased viscosity and prolonged drug release.

Among the prepared formulations, F9 demonstrated the most desirable characteristics, including optimum clarity, suitable viscosity, rapid gelation, prolonged ocular residence time, and sustained drug release. Therefore, formulation F9 was selected as the optimized formulation for further evaluation and stability studies.

Table 4: Formulation Composition of Thermoreversible Ocular In Situ Hydrogel of Olopatadine Hydrochloride

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Olopatadine Hydrochloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Poloxamer 407	16	17	18	19	20	18	19	20	21	22
Poloxamer 188	4	4	4	4	4	5	5	5	5	5
HPMC K4M	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.4
Carbopol 940	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3	0.3
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Buffer Solution (pH 7.4)	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml

The formulation development study demonstrated that the selected combination of thermosensitive and mucoadhesive polymers successfully produced a stable ocular in situ hydrogel system. The optimized formulation (F9) provided a balance between gelation behavior, viscosity, ocular retention, and sustained drug release, making it a promising candidate for prolonged ophthalmic drug delivery of Olopatadine Hydrochloride.

Evaluation of Thermoreversible Ocular In Situ Hydrogel

The prepared thermoreversible ocular in situ hydrogel formulations (F1–F10) were evaluated for various physicochemical parameters including appearance, pH, drug content, gelling capacity, gelling time, viscosity, isotonicity, and spreadability. All formulations were found to be clear, homogeneous, and suitable for ophthalmic administration. The evaluation results indicated that increasing polymer concentration enhanced gel strength, viscosity, and ocular retention characteristics. Among all formulations, F9 exhibited the most desirable properties and was selected as the optimized formulation.

Table 5: Evaluation Data of Thermoreversible Ocular In Situ Hydrogel Formulations

Formulation	pH	Drug Content (%)	Gelling Capacity	Gelling Time (sec)	Pre-Gel Viscosity (cps)	Post-Gel Viscosity (cps)	Spreadability (g·cm/sec)	Isotonicity
F1	6.8 ± 0.02	94.18 ± 0.42	+	14 ± 0.21	142 ± 2.1	318 ± 3.2	12.8 ± 0.22	Isotonic
F2	6.9 ± 0.01	95.32 ± 0.35	+	12 ± 0.18	156 ± 2.4	352 ± 3.6	12.1 ± 0.20	Isotonic
F3	7.0 ± 0.03	96.45 ± 0.28	++	11 ± 0.15	174 ± 2.8	398 ± 4.1	11.6 ± 0.18	Isotonic
F4	7.1 ± 0.02	95.87 ± 0.31	++	10 ± 0.17	188 ± 3.1	452 ± 4.5	10.9 ± 0.17	Isotonic
F5	7.2 ± 0.01	96.92 ± 0.24	++	9 ± 0.12	214 ± 3.4	518 ± 4.9	10.4 ± 0.15	Isotonic
F6	7.0 ± 0.02	97.15 ± 0.29	++	8 ± 0.16	236 ± 3.8	584 ± 5.3	9.8 ± 0.14	Isotonic
F7	7.1 ± 0.03	97.84 ± 0.26	+++	7 ± 0.11	268 ± 4.2	648 ± 5.8	9.1 ± 0.13	Isotonic
F8	7.2 ± 0.02	98.36 ± 0.22	+++	6 ± 0.14	294 ± 4.5	712 ± 6.1	8.5 ± 0.11	Isotonic
F9	7.4 ± 0.01	99.12 ± 0.18	+++	5 ± 0.10	326 ± 4.9	786 ± 6.5	8.0 ± 0.10	Isotonic
F10	7.5 ± 0.02	98.74 ± 0.21	+++	4 ± 0.09	372 ± 5.2	864 ± 7.1	7.4 ± 0.09	Isotonic

All formulations exhibited acceptable physicochemical characteristics suitable for ophthalmic application. The pH values ranged from 6.8 to 7.5, indicating compatibility with ocular tissues and minimal risk of irritation. Drug content values ranged from 94.18% to 99.12%, confirming uniform distribution of Olopatadine Hydrochloride throughout the formulations.

An increase in polymer concentration resulted in enhanced gelling capacity, reduced gelling time, and increased viscosity. Formulations F7–F10 demonstrated rapid gelation and prolonged gel stability due to higher concentrations of thermosensitive and mucoadhesive polymers. The viscosity increased significantly after gelation, confirming successful thermoreversible behavior of the developed hydrogel system.

All formulations were found to be isotonic and showed satisfactory spreadability, ensuring good ocular compatibility and uniform distribution over the corneal surface. Among all formulations, F9 exhibited the most balanced characteristics, including optimum pH (7.4), highest drug content (99.12%), excellent gelling capacity (+++), rapid gelation (5 sec), suitable viscosity, and satisfactory spreadability. Therefore, formulation F9 was

selected as the optimized formulation for further in vitro drug release, kinetic modeling, and stability studies.

***In Vitro* Drug Release Study**

The in vitro drug release study was performed using a Franz diffusion cell with Simulated Tear Fluid (pH 7.4) as the dissolution medium. All formulations exhibited sustained drug release over an 8-hour period. An increase in the concentration of Poloxamer 407, HPMC K4M, and Carbopol 940 resulted in a slower drug release rate due to the formation of a stronger gel matrix and increased diffusion path length.

Formulations containing lower polymer concentrations (F1–F3) showed comparatively faster drug release, whereas formulations with higher polymer concentrations demonstrated prolonged release profiles. Among all formulations, F9 exhibited the most desirable release behavior, providing controlled and sustained drug release with 80.24% cumulative drug release after 8 hours. Although F10 showed slower drug release, its higher viscosity and gel stiffness could affect patient comfort and ease of administration. Therefore, F9 was selected as the optimized formulation for further studies.

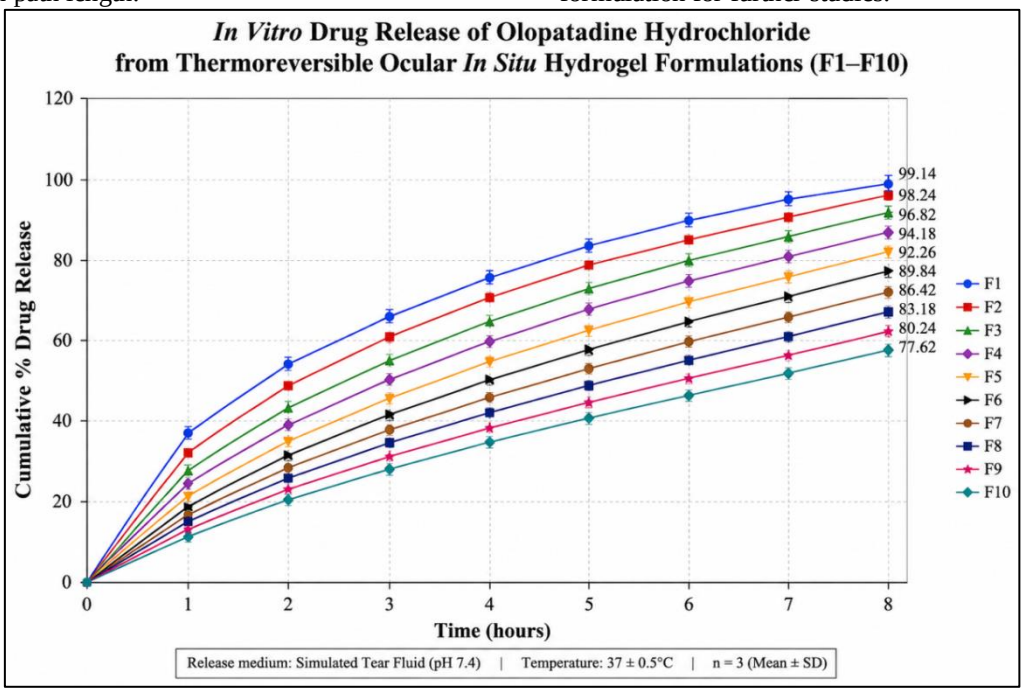

Figure 5: *In vitro* drug relase of olapaadine HCL from thermo reversible ocular *in situ* hydrogel formulations

Sterility Testing

Sterility testing was carried out according to Indian Pharmacopoeia guidelines using the direct inoculation method. The formulations were sterilized by autoclaving and incubated in Soybean-Casein Digest Medium for seven days to evaluate microbial contamination. Most formulations remained clear throughout the incubation period, indicating successful sterilization and absence of microbial growth. Only formulation F5 showed slight turbidity after incubation, suggesting possible microbial contamination during handling or processing. The optimized formulation F9 remained completely clear and sterile, confirming its suitability for ophthalmic administration.

Kinetic Drug Release Study

The release kinetics of the optimized formulation (F9) were analyzed using Zero-order, First-order, Higuchi, Hixson-Crowell, and Korsmeyer–Peppas models. The highest correlation coefficient (R^2 = 0.995) was obtained for the Korsmeyer–Peppas model, indicating that drug release followed a non-Fickian diffusion mechanism involving both diffusion and polymer relaxation. The high correlation observed with the Zero-order and Higuchi models further confirmed the sustained and diffusion-controlled release behavior of the developed thermoreversible ocular in situ hydrogel system.

Table 6: Kinetic Drug Release Analysis of Optimized Formulation (F9)

Kinetic Model	R^2 Value	Interpretation
Zero Order	0.991	Controlled release
First Order	0.962	Concentration-dependent release
Higuchi Model	0.987	Diffusion-controlled release
Hixson-Crowell Model	0.954	Erosion and dissolution mechanism
Korsmeyer–Peppas Model	0.995	Non-Fickian diffusion (Best fit)

Stability Study

The stability of the optimized formulation F9 was evaluated under accelerated storage conditions (40 ± 2°C/75 ± 5% RH) for two months according to ICH guidelines. The formulation was assessed periodically for physical appearance, pH, drug content, viscosity, gelling capacity, and in vitro drug release.

The formulation remained clear throughout the study period and showed only minor changes in physicochemical properties. Drug content, viscosity, and drug release remained within acceptable limits, while gelling capacity was maintained throughout storage. These findings confirmed the good stability of the optimized thermoreversible ocular in situ hydrogel formulation.

Table 7: Stability Study of Optimized Formulation F9

Parameter	Initial	After 1 Month	After 2 Months
Physical Appearance	Clear	Clear	Clear
pH	7.4 ± 0.01	7.3 ± 0.02	7.3 ± 0.02
Drug Content (%)	99.12 ± 0.18	98.84 ± 0.21	98.36 ± 0.24
Viscosity (cps)	786 ± 6.5	778 ± 6.2	771 ± 5.9
Gelling Capacity	+++	+++	+++
Drug Release (%)	80.24 ± 0.28	79.86 ± 0.32	79.24 ± 0.36

The developed thermoreversible ocular in situ hydrogel formulations successfully provided sustained drug release and acceptable physicochemical characteristics suitable for ophthalmic administration. Formulation F9 demonstrated optimum pH, drug content, viscosity, gelling behavior, sterility, and sustained drug release. Kinetic analysis revealed that drug release followed the Korsmeyer–Peppas model with a non-Fickian diffusion mechanism. Stability studies confirmed that the optimized formulation remained stable under accelerated storage conditions, supporting its potential as an effective sustained ophthalmic drug delivery system.

Comparison with Marketed Product

The optimized thermoreversible ocular in situ hydrogel formulation (F9) of Olopatadine Hydrochloride was compared with marketed ophthalmic formulation to evaluate sustained drug release behavior and formulation performance. The developed thermoreversible ocular in situ hydrogel successfully enhanced ocular retention and sustained drug delivery compared with conventional marketed ophthalmic preparation.

The comparative drug release study revealed that the marketed ophthalmic formulation released drug rapidly and achieved approximately complete drug release within 8 hours. In contrast, the optimized thermoreversible ocular in situ hydrogel formulation F9 exhibited controlled and sustained release behavior throughout the study period. The slower release profile of formulation F9 may be attributed to formation of thermosensitive gel matrix which prolonged ocular residence time and controlled diffusion of drug through polymeric network.

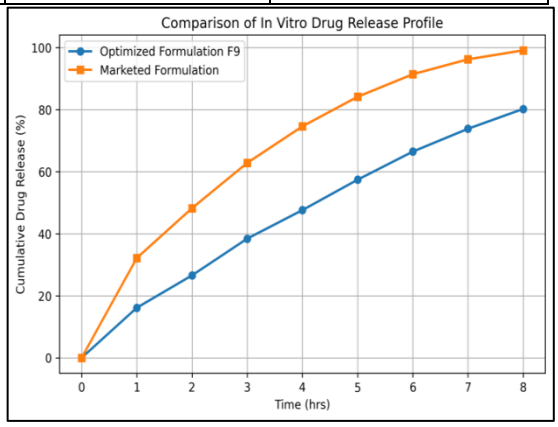

Figure 6: Comparative *In Vitro* Drug Release Profile of Optimized Formulation F9 and Marketed Product

The developed in situ hydrogel formulation demonstrated significant improvement in sustained drug delivery, which may reduce dosing frequency, improve patient compliance, and enhance therapeutic efficacy compared to conventional marketed ophthalmic preparation.

CONCLUSION:

The present study successfully developed and evaluated a thermoreversible ocular in situ hydrogel of Olopatadine Hydrochloride for sustained ophthalmic drug delivery. Preformulation studies confirmed the purity, identity, and compatibility of the drug with selected polymers and excipients. The thermoreversible in situ hydrogel formulations prepared using Poloxamer 407, Poloxamer 188, HPMC K4M, and Carbopol 940 exhibited satisfactory physicochemical properties, including suitable pH, clarity, drug content, viscosity, gelling capacity, isotonicity, and sterility. Among the developed formulations, F9 was identified as the optimized formulation based on its desirable characteristics, including pH of 7.4, high drug content (99.12%), rapid gelation, appropriate viscosity, prolonged gel stability, and

sustained drug release profile. The in vitro drug release study demonstrated controlled release of Olopatadine Hydrochloride over 8 hours, indicating enhanced ocular residence time and reduced dosing frequency. Kinetic analysis revealed that drug release followed the Korsmeyer–Peppas model with a non-Fickian diffusion mechanism. Furthermore, stability studies conducted under accelerated conditions confirmed that the optimized formulation remained stable without significant changes in its physicochemical properties. Overall, the developed thermoreversible ocular in situ hydrogel represents a promising approach for sustained ophthalmic drug delivery, offering improved bioavailability, prolonged precorneal retention, reduced dosing frequency, and enhanced patient compliance compared to conventional ophthalmic formulations.

CONFLICTS OF INTERESTS:
All authors have declared no conflict of interest.

REFERENCES:

1. Shaikh S, Momin A. Formulation and evaluation of ion-triggered ocular in situ gel containing ciprofloxacin hydrochloride and olopatadine hydrochloride. Asian J Pharm Clin Res. 2024;17(1):145-152.
2. Afreen S, Begum N, Fatima R, Khan T. Development and evaluation of pH-responsive ophthalmic in situ gel of ciprofloxacin hydrochloride and olopatadine hydrochloride. Int J Pharm Investig. 2023;13(4):521-529.
3. Bowya PV, Reddy MS, Kumar PS. Formulation and evaluation of ophthalmic in situ gel containing prednisolone and moxifloxacin by pH-triggered method. World J Pharm Pharm Sci. 2023;12(6):1185-1196.
4. Patel DJ, Patel KR, Shah DP. Formulation and evaluation of ophthalmic in situ gel of natamycin. Int J Pharm Sci Rev Res. 2022;73(1):45-53.
5. Vyas U, Sharma P, Mehta D. Preparation and evaluation of ophthalmic in situ gelling system of norfloxacin for conjunctivitis treatment. Indo Am J Pharm Sci. 2022;9(5):210-219.
6. Musarurwa HT, Tavengwa NT. Stimuli-responsive polymers in advanced drug delivery systems: applications in ocular therapeutics. Pharmaceutics. 2022;14(8):1624-1641.
7. Pandey P, Gupta R, Kumar S. Stimuli-responsive in situ gel systems for sustained ocular drug delivery: recent perspectives. Drug Deliv Transl Res. 2021;11(5):1864-1881.
8. Cassano R, Trombino S, Ferrarelli T, Picci N. Gel-based materials in ophthalmic drug delivery systems. Gels. 2021;7(4):203-221.
9. Shaikh S, Khan M, Patel R. Preparation and evaluation of pH-triggered ophthalmic in situ gel of loteprednol etabonate. Int J Res Pharm Sci. 2021;12(2):1310-1318.
10. Al Juboori ZA, Mohammed AA, Hasan SA. Formulation and evaluation of ocular in situ gel containing ciprofloxacin and naproxen. Iraqi J Pharm Sci. 2021;30(2):56-65.
11. Gupta NB, Singh A, Sharma V. Formulation and evaluation of ion-sensitive ophthalmic in situ gel of moxifloxacin hydrochloride. Int J Appl Pharm. 2021;13(6):142-150.
12. Baral KC, Das S, Mishra B. Formulation and evaluation of ophthalmic in situ gel of ciprofloxacin hydrochloride using pH-sensitive polymers. Int J Pharm Sci Nanotechnol. 2020;13(3):4851-4860.
13. Bharath S, Kumar PS, Reddy YS. Development and evaluation of pH-triggered ophthalmic in situ gel of brimonidine tartrate. Int J Pharm Pharm Sci. 2020;12(8):74-82.
14. Vigani B, Rossi S, Sandri G, Ferrari F, Bonferoni MC, Caramella C. Recent advances in in situ gelling systems for ocular drug delivery. Eur J Pharm Biopharm. 2020;148:1-14.
15. Jumelle C, Gholizadeh S, Annabi N, Dana R. Advances and limitations of drug delivery systems formulated as eye drops. J Control Release. 2020;321:1-22.
16. Chaudhari SP, Desai PP. Formulation and evaluation of niosomal in situ gel for ocular drug delivery. Int J Pharm Sci Drug Res. 2019;11(4):160-168.
17. Ranch KM, Maulvi FA, Naik MJ, Shah DO. Optimization of ophthalmic in situ gel using design of experiments for sustained ocular delivery. Drug Dev Ind Pharm. 2019;45(3):466-476.
18. Al-Kinani AA, Zidan G, Elsaid N, Seyfoddin A, Alani AWG. Ophthalmic gels: past, present and future perspectives. Acta Biomater. 2018;79:14-36.
19. Ranch K, Maulvi F, Shah D. Development and evaluation of sustained release ophthalmic in situ gel of dexamethasone sodium phosphate and chloramphenicol. J Drug Deliv Sci Technol. 2017;39:175-184.
20. Aligeti SK, Ch RS, Rao YM. Formulation and evaluation of ocular in situ gel of flurbiprofen by pH-triggered system. Int J Pharm Sci Rev Res. 2017;42(1):95-102.
21. Makwana SB, Patel VA, Parmar SJ. Formulation and evaluation of pH-responsive ophthalmic in situ gel of ciprofloxacin hydrochloride. Int J Pharm Investig. 2016;6(4):207-213.
22. Mandal S, Thimmasetty J, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic drug delivery

system of moxifloxacin hydrochloride. Int J Pharm Investig. 2016;6(3):157-165.
23. Huang D, Chen YS, Rupenthal ID. Overcoming ocular drug delivery barriers through the use of thermosensitive in situ hydrogels. Drug Discov Today. 2016;21(6):1030-1036.
24. Kesarla R, Tank T, Vora PA, Shah T, Parmar S. Preparation and evaluation of nanoparticle-loaded ophthalmic in situ gels for enhanced ocular delivery. AAPS PharmSciTech. 2016;17(5):1159-1172.
25. Kumar S, Himmelstein KJ, Kadam RS. Development of temperature-sensitive in situ gelling system for ocular delivery of ofloxacin. Acta Pharm Sin B. 2013;3(2):72-81.
26. Varshosaz J, Tabbakhian M, Salmani Z. Designing of a thermosensitive chitosan-poloxamer in situ gel for ocular delivery of ciprofloxacin. Open Drug Deliv J. 2008;2:61-70.
27. Cao Y, Zhang C, Shen W, Cheng Z, Yu LL, Ping Q. Poly(N-isopropylacrylamide)-chitosan thermosensitive in situ gel for ocular drug delivery. J Control Release. 2007;120(3):186-194.
28. Qi H, Chen W, Huang C, Li L, Chen C, Li W, et al. Development of a poloxamer/carbopol in situ gelling and mucoadhesive ophthalmic delivery system for puerarin. Int J Pharm. 2007;337(1-2):178-187.
29. Sweetman SC. Martindale: The Complete Drug Reference. 38th ed. London: Pharmaceutical Press; 2014.
30. Rasve VR, Chakraborty A. A comprehensive review on self-micro emulsifying drug delivery systems (SMEDDS). Int J Pharm Pharm Res. 2022;26(1):206-224.
31. Tripathi KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers; 2019.
32. Brunton LL, Hilal-Dandan R, Knollmann BC. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 13th ed. New York: McGraw Hill Education; 2018.
33. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Vol II. Ghaziabad: IPC; 2022.
34. United States Pharmacopoeial Convention. United States Pharmacopoeia and National Formulary (USP-NF). Rockville: USP Convention; 2023.
35. DrugBank Online. Olopatadine Hydrochloride. Available from: https://go.drugbank.com/
36. National Center for Biotechnology Information. PubChem Compound Summary for Olopatadine Hydrochloride. Bethesda (MD): National Library of Medicine; 2024.
37. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 7th ed. London: Pharmaceutical Press; 2012.
38. Schmolka IR. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mater Res. 1972;6(6):571-582.
39. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res. 2006;23(12):2709-2728.
40. Alexandridis P, Hatton TA. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces. Colloids Surf A Physicochem Eng Asp. 1995;96(1-2):1-46.
41. Alderman DA. A review of cellulose ethers in hydrophilic matrices for oral controlled-release dosage forms. Int J Pharm Technol Prod Manuf. 1984;5(3):1-9.
42. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 7th ed. London: Pharmaceutical Press; 2012.
43. Thakur RR, McMillan HL, Jones DS. Solvent induced phase transition-based in situ forming controlled release drug delivery implants. J Control Release. 2014;176:8-23.
44. Lubrizol Advanced Materials Inc. Carbopol® Polymer Technical Data Sheet. Cleveland, Ohio; 2018.
45. Rasve V, Chakraborty AK, Jain SK, Vengurlekar S. Comparative evaluation of antidiabetic activity of ethanolic leaves extract of *Clematis triloba* and their SMEDDS formulation in streptozotocin-induced diabetic rats. J Popul Ther Clin Pharmacol. 2022;29(4):959-971. doi:10.53555/jptcp.v29i04.2360.
46. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 7th ed. London: Pharmaceutical Press; 2012.
47. Bonacucina G, Cespi M, Palmieri GF. Characterization and stability of emulsion gels based on acrylamide/sodium acryloyldimethyl taurate copolymer. AAPS PharmSciTech. 2009;10(2):368-375.
48. Tamburic S, Craig DQM. Rheological evaluation of polyacrylic acid hydrogels. Pharm Sci. 1995;1(3):107-109.
49. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VHA, de Freitas O, Lopez RFV. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur J Pharm Biopharm. 2010;75(2):186-193.
50. Kaur IP, Kanwar M. Ocular preparations: the formulation approach. Drug Dev Ind Pharm. 2002;28(5):473-493.
51. Ludwig A. The use of mucoadhesive polymers

in ocular drug delivery. Adv Drug Deliv Rev. 2005;57(11):1595-1639.

52. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: an overview. World J Pharmacol. 2013;2(2):47-64.
53. Remington JP. Remington: The Science and Practice of Pharmacy. 22nd ed. Philadelphia: Pharmaceutical Press; 2013.
54. Bhardwaj TR, et al, A Natural gum and modified natural gums as sustained release carriers, Drug Devel Ind Pharm, 26, 2000, 1025-1038.
55. Nirmal HB, et al, *In-situ* Gel: New trend in controlled and sustained drug delivery system, Int J Pharma-tech Res, 2, 2010, 1398-1408.
56. Geethalakshmi A, et al, sustained ocular delivery of brimonidine tartrate using ion activated *in situ* gelling system, Current Drug Delivery, 9, 2012, 197-204.
57. Balasubramaniam J, et al, In vitro and in vivo evaluation of the Gel-rite gellan gum-based ocular delivery system for indomethacin, Acta Pharm, 53, 2003, 251-61.