RESEARCH PAPER

A Novel In Situ Gel-Based Nanoparticle System for Controlled Release of Levofloxacin in Bacterial Infections

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ABSTRACT

Objective: Develop and evaluate levofloxacin-loaded polymeric nanoparticles incorporated into a thermosensitive in-situ gel for sustained ocular drug delivery.

Methods: PLGA and Eudragit RL100, stabilised by polyvinyl alcohol, were used to prepare levofloxacin-loaded nanoparticles using the oil-in-water emulsification solvent evaporation method. The nanoparticles were incorporated into a poloxamer-based thermosensitive in-situ gel by the cold dispersion method. Formulations (F1 to F6) were evaluated for their physicochemical properties, particle size, zeta potential, drug loading, encapsulation efficiency, pH, gelation behaviour, viscosity, SEM, XRD, and in vitro drug release.

Results: F3 showed the best performance with a particle size of 5.62 nm, zeta potential of −18 mV, and encapsulation efficiency of 96.265%. The formulation displayed a pH of 7.25, a gelation time of 35 seconds, and an ideal gelling temperature of 37 °C. XRD and SEM indicated uniform spherical morphology, while SEM indicated reduced crystallinity. Studies conducted in vitro and ex vivo demonstrated sustained drug release (90% versus 87% at 6 hours).

Conclusion: F3 nanoparticle in-situ gel formulations demonstrated suitable physicochemical characteristics and sustained drug release, indicating their potential as an effective ocular drug delivery system.

Keywords: Levofloxacin, polymeric nanoparticles, in-situ gel, ocular drug delivery, sustained release, PLGA.

How to cite this article: Mohanty D, Giri S, Panda S, Kumar P. A Novel In Situ Gel-Based Nanoparticle System for Controlled Release of Levofloxacin in Bacterial Infections. Int J Drug Deliv Technol. 2026;16(14s): 289-304. DOI: 10.25258/ijddt.16.14s.33

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Drug delivery to the eye remains a significant challenge due to the unique protective mechanisms of the eye, such as rapid tear turnover, blinking, nasolacrimal drainage, and limited corneal permeability. It is common for these physiological barriers to result in poor ocular bioavailability and reduced therapeutic efficacy of conventional ophthalmic dosage forms, such as eye drops and suspensions. A small fraction of drugs administered to the eye reaches the intraocular tissues, while the remaining portions are rapidly eliminated from the precornea. Due to this, there is a continuous need to develop advanced ocular drug delivery systems capable of enhancing precorneal residence time, improving corneal permeation, and providing sustained drug release(1–3).

Levofloxacin is a fluoroquinolone antibiotic that is widely used for treating bacterial eye infections, including conjunctivitis, keratitis, and corneal ulcers. In spite of the fact that conventional levofloxacin ophthalmic solutions are effective, they must be administered frequently due to rapid precorneal loss and short contact times with ocular tissues. In addition to reducing patient compliance, frequent dosing can also disturb the concentration of the drug at the site of action. In view of these considerations, levofloxacin may be more effectively delivered to the eye through a sustained and targeted delivery system(4).

Due to their ability to improve drug stability, enhance corneal penetration, and provide controlled drug release, nanoparticle-based drug delivery systems are proving to be promising carriers for ocular administration(5). Due to their biocompatibility, biodegradability, and ability to encapsulate drugs, polymeric nanoparticles, especially those prepared with biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), have become widely used in the pharmaceutical industry(6). Because of its permeability characteristics, Eudragit RL100 is also a useful polymer for controlled release applications(2). An in-situ gelling system that incorporates drug-

loaded nanoparticles can further improve ocular retention by allowing the formulation to be administered as a liquid that transforms into a gel under physiological conditions(7–11).

For ophthalmic drug delivery, thermosensitive in-situ gels based on poloxamers are particularly attractive(12–15). These systems remain liquid at room temperature, allowing for easy instillation, and undergo a sol-gel transition at the corneal surface, prolonging their residence time there. The use of a combined system comprised of polymeric nanoparticles dispersed in a thermosensitive in-situ gel might provide the dual benefits of sustained release and improved ocular retention(1,9,16–20).

The current study aims to develop and evaluate levofloxacin-loaded polymeric nanoparticles incorporated into an in-situ gelling system for sustained and targeted delivery to the eye. For the purpose of identifying the optimal formulation for ophthalmic use, physicochemical properties, compatibility, particle size, zeta potential, encapsulation efficiency, pH, gelation behaviour, viscosity, surface morphology, crystallinity, drug release in vitro, and in-vivo permeation were analysed.

MATERIALS

Levofloxacin Hemihydrate (CAS No. 138199-71-0) was purchased from Dhamtec Pharma Pvt. Ltd., Maharashtra, India. PLGA (CAS No. 26780-50-7), Poloxamer 407 (CAS No. 9003-11-6), Poloxamer 188 (CAS No. 9003-11-6), and Eudragit RL 100 (CAS No. 33434-24-1) were procured from Alpha Chemika Pvt. Ltd., Mumbai, Maharashtra, India. Polyvinyl alcohol (PVA) (CAS No. 9002-89-5) was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Dichloromethane (CAS No. 75-09-2) and benzalkonium chloride (CAS No. 63449-41-2) were purchased from SRL Chemicals Pvt. Ltd., India. Potassium dihydrogen phosphate (CAS No. 7778-77-0), sodium chloride (CAS No. 7647-14-5), and potassium chloride (CAS No. 7447-40-7) were supplied by Nice Pharmaceuticals Pvt. Ltd., India. Sodium hydroxide pellets (CAS No. 1310-73-2) were obtained from Advent Pharmaceuticals Pvt. Ltd., India. Potassium chloride (CAS No. 7447-40-7) was also sourced from Fisher Inorganics and Aromatics Ltd., India. Sodium bicarbonate (CAS No. 144-55-8) was purchased from Spectrum Chemical Pvt. Ltd., India. All chemicals and reagents used in the study were of analytical grade and were used without further purification(21).

METHODS

Organoleptic characterisation and Solubility studies

Organoleptic evaluation is a preliminary qualitative assessment performed during preformulation studies to examine the observable physical characteristics of a drug substance, such as colour, appearance, odour, and texture. The organoleptic properties of a small quantity of the pure drug were assessed by visual inspection on a clean white surface. The organoleptic properties of a small quantity of the pure drug were assessed by visual inspection on a clean white surface. Levofloxacin hemihydrate has been evaluated for its solubility in various solvents. In this study, distilled water, simulated tear fluid (pH 7.4), phosphate buffer (pH 6.8), and ethanol were used as solvents(22,23)

UV-vis spectrophotometric analysis

A primary stock solution containing a concentration of 1000 mg/ml was prepared from 10 mg of Levofloxacin accurately weighed and dissolved in 10 ml of distilled water. The stock solution was withdrawn in 2.5 ml portions and transferred to a 25 ml volumetric flask, and the volume was topped up with distilled water to obtain a working standard solution of 100 mg/ml. We prepared working solutions in aliquots of 0.10 ml, 0.20 ml, 0.4 ml, 0.8 ml, and 1.0 ml and transferred them into volumetric flasks of 0.1 ml, 0.22 ml, 0.4 ml, 0.8 ml, and 1.0 ml, respectively. A solution was prepared by diluting the flasks with distilled water to produce solutions having concentrations of 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, and 10µg/ml, respectively. The absorbance of both solutions was determined using a UV-Visible spectrophotometer at the predetermined maximum degree of Levofloxacin, and distilled water was used as a blank. The recorded absorbance values were plotted against concentration to construct a calibration curve(24).

Drug–Excipient Compatibility Studies

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy was used to examine the potential interactions between Levofloxacin hemihydrate and selected excipients. FT-IR samples were prepared using potassium bromide pellets (KBr). As part of this method, a small amount of the pure drug and the physical mixture of the drug with excipients are blended with dry potassium bromide and compressed into pellets. After preparing the pellets, they were analysed with an FT-IR spectrophotometer (Bruker Alpha, Bruker Corporation, Germany) over a scanning range of 4000 to 400 cm1. The obtained

spectra were evaluated to identify characteristic functional groups of the drug and to identify any possible chemical interactions with the excipients(25).

Differential Scanning Calorimetry (DSC) Study

It was determined that the thermal behaviour of Levofloxacin hemihydrate and the compatibility of the formulation excipients could be further assessed by differential scanning calorimetry. Samples of the pure drug and the drug-excipient mixtures were weighed accurately and then analysed under a nitrogen atmosphere using a DSC instrument. To record the thermal transitions, including the melting endotherm of the drug, the samples were heated at a constant rate over a suitable temperature range. As a result, thermograms were compared to determine if there was any change in melting point or thermal pattern, which could indicate interactions between the drug and excipients.

Preparation of polymeric drug-loaded nanoparticles(22,24,26,27)

Levofloxacin-loaded PLGA nanoparticles were prepared by emulsifying oil in water (O/W). A solution of 750 mg polyvinyl alcohol (PVA) was prepared by heating it at 70–80°C under magnetic stirring (300–400 rpm) until a clear solution was obtained. The aqueous phase was formed by dissolving 750 mg of levofloxacin hemihydrate in water after letting it cool to room temperature. A continuous stirring method was used to dissolve 1000 mg of PLGA in 30 mL of dichloromethane (DCM). A coarse emulsion was formed by adding the organic solution dropwise to an ice bath maintained with constant stirring. Further processing of this emulsion involved high-speed homogenization (10,000–15,000 rpm for 5 minutes) followed by probe sonication (Cole Parmer) to obtain nanoparticles with reduced particle size. As a last step, the nanosuspension was stabilised with 0.5% lactose as a cryoprotectant and lyophilised (SP Scientific Benchtop Pro) at 40°C to obtain a dry nanoparticle powder suitable for incorporation into an in-situ gel system for ophthalmology.

Particle Size Analysis

Levofloxacin-loaded nanoparticles were analysed using a dynamic light scattering technique (DLS) and a Zetasizer (Malvern Version 8.02, Malvern Panalytical, Worcestershire, UK). The nanoparticle dispersion was diluted with distilled water and gently mixed before analysis in order to ensure uniform dispersion and minimize particle aggregation and multiple scattering. An approximate count rate of 25 kcps was maintained throughout the measurements at a controlled temperature of 25°C. Analyses were conducted on the drug-loaded nanoparticles prior to their incorporation into the ophthalmic in situ gel formulation.

Zeta Potential Measurement

Zeta potentials of Levofloxacin-loaded nanoparticles were determined using a Zetasizer (Malvern Ver. 8.02, Malvern Panalytical, Worcestershire, UK) in order to evaluate the surface charge and stability of the nanoparticle system. To prepare the nanoparticle dispersion for analysis, it was diluted appropriately with distilled water, which was used as the dispersing medium. Measurements were performed at a temperature of 25 °C and a count rate of approximately 25 kcps. To assess the stability and possible interactions between nanoparticles and in situ ophthalmic gel, this evaluation was performed on the nanoparticle formulation before its incorporation into the gel.

DRUG LOADING AND ENCAPSULATION EFFICIENCY

The drug loading (DL) and encapsulation efficiency (EE) of Levofloxacin-loaded nanoparticles were determined by measuring the amount of free (unencapsulated) drug in the supernatant. The nanoparticle dispersion was centrifuged at 15,000 rpm for 30 minutes at 4 °C using a high-speed refrigerated centrifuge. A supernatant containing the unentrapped drug was carefully collected and diluted with phosphate buffer (pH 7.4). Levofloxacin concentrations were measured with a UV-visible spectrophotometer at the predetermined maximum (approximately 287 nm) in comparison with a blank. During formulation, the amount of free drug present in the supernatant was subtracted from the total amount of drug initially added.

As a result of applying the following equations, we were able to calculate the encapsulation efficiency and drug loading capacity:

$$Encapsulation\ efficiency\% = \frac{Total\ drug\ added - Free\ drug\ in\ Supernatant}{Total\ drug\ added} \times 100$$

$$Drug\ loading\% = \frac{Amount\ of\ drug\ encapsulated}{Total\ weight\ of\ Nanoparticles} \times 100$$

MICROSCOPIC IMAGE OF PREPARED NANOPARTICLES

The prepared nanoparticles were examined under the microscope to determine their physical appearance, shape, and dispersion. Nanoparticle suspension was

placed on a clean glass slide and covered with a coverslip. Particles were then viewed under an optical microscope at appropriate magnifications using an optical microscope.

PROCEDURE FOR THE PREPARATION OF THE FINAL MEDICATED FORMULATION(25,28–32)

The cold dispersion method was used to prepare a thermosensitive in-situ gel base. Initially, Poloxamer 407 was added to cold distilled water (4–8 °C) under continuous magnetic stirring (400–600 rpm) to ensure uniform dispersion and prevent lump formation. Poloxamer 188 (2 g) was then added to the solution and stirred until it was fully dissolved. A clear and homogeneous gel base was obtained by keeping the dispersion under refrigeration (@4°C) overnight to allow complete hydration of the polymers. To obtain a uniform suspension, lyophilised PLGA nanoparticles containing levofloxacin (equivalent to 15 mg of drug) were dispersed in cold phosphate buffer solution (PBS) with gentle stirring (300–400 rpm). As a preservative, benzalkonium chloride dissolved in cold PBS was slowly added to the dispersion. To maintain low temperatures and ensure uniform distribution of the nanoparticle suspension, the nanoparticle suspension was gradually incorporated into the pre-cooled poloxamer gel base with continuous stirring in an ice bath. Afterwards, the mixture was stirred for another 20–30 minutes in order to achieve complete homogenization and then stored at 4°C for 12–24 hours to allow the formulation to stabilise. The optimised preparation remained a free-flowing liquid at room temperature (25°C) and transformed into a gel at ocular temperature (37°C), thereby enhancing the precorneal residence time and facilitating sustained drug release.

Table 1: Composition of Formulations for the Preparation of Levofloxacin-Loaded Nanoparticle In-Situ Gel

Ingredients Used	F1	F2	F3	F4	F5	F6
Levofloxacin hemihydrate	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg
PVA	250 mg	500 mg	750 mg	250 mg	500 mg	750 mg
PLGA	250 mg	500 mg	1000 mg	----	----	----
Eudragit RL 100	----	----	----	250	500	1000
Poloxamer 407	750 mg	500 mg	250 mg	750 mg	500 mg	250 mg
Benzalkonium Chloride	q. s	q. s	q. s	q. s	q. s	q. s
Distilled Water	q. s	q. s	q. s	q. s	q. s	q. s

pH Measurement

Each formulation's pH was determined using a digital pH meter (Systronics µ-PH-335, Systronics India Ltd., Ahmedabad, India). Simulated tear fluid of pH 4.0, 7.0, and 9.0 were used to calibrate the instrument. The pH readings of the prepared formulations were then recorded at a controlled temperature of 25 ± 0.5 °C.

Preparation of Simulated tear fluid (pH 7.4)

Simulated tear fluid (STF) was prepared with a pH of 7.4 to mimic the ocular environment. An appropriate volume of distilled water was used to dissolve sodium chloride, potassium chloride, sodium bicarbonate, and calcium chloride under continuous stirring. After the solution was prepared, the pH was adjusted to 7.4 with sodium hydroxide or hydrochloric acid. After the solution was filled up with distilled water to the required level, the simulated tear fluid was stored in a clean, airtight container for further experimentation.

Determination of the Gelling Capacity of Nano In-situ gel base

In situ gel formulations containing nanoparticles were evaluated by observing the sol-gel transition when the formulations were exposed to simulated tear fluid (STF, pH 7.4). To simulate ocular conditions, 2 mL of simulated tear fluid at 37 °C was placed in a glass vial. The gelation behaviour of the nano in-situ gel formulation was then observed by adding a drop carefully into the medium and observing the gelation process visually. A record of the time required for the gel to form and the duration during which it remained stable was kept. The gelling ability was evaluated qualitatively based on the rate at which the gel formed and the persistence of the gel structure. The formulations showing rapid gelation and prolonged gel integrity were deemed to possess good gelling capacity and were selected for further evaluation.

Determination of the Gelling Temperature of Nano In-situ Gel Base

In-situ gel formulations loaded with nanoparticles were tested using a simple visual inspection method. The formulation was transferred into a glass vial and placed in a temperature-controlled water bath while it was gently stirred continuously. Gradually increasing

the temperature of the system at a constant rate, the formulation was carefully observed. The gelling temperature is the temperature at which the formulation transforms from a free-flowing liquid to a semi-solid gel upon tilting the vial. An evaluation of this parameter is essential for determining the thermosensitive behaviour of the in-situ gel system and ensuring that gelation takes place at a temperature close to that of the human eye (approximately 34 to 37ºc).

Determination of the Isotonicity of Nano In-situ Gel Base

A human blood sample was used to assess the isotonicity of the formulation. To prevent coagulation, blood was collected in an EDTA-containing vial. The in-situ gel forming solution was poured into a china dish, followed by a portion of the collected blood. A gentle agitation was performed in order to ensure that the blood and formulation are properly mixed. After mixing, a small amount of blood was drawn up to the 0.5 mark of an RBC pipette and diluted with RBC diluting fluid. On a clean glass slide, a drop of the diluted sample was placed and examined under a microscope. Red blood cells (RBCs) were examined under a microscope for any morphological changes such as crenation (cell shrinkage), swelling, or hemolysis (cell rupture). As a result of the absence of such changes, the formulation was isotonic and compatible with blood cells.

Determination of the Viscosity of Nano In-situ Gel Base

A Brookfield viscometer (AMETEK Brookfield) was used to determine the viscosity of the formulation under two different experimental conditions: (1) varying rotational speeds at a constant temperature and (2) varying temperatures at a constant rotational speed. A suitable beaker was used for measuring 50 mL of the prepared formulation. A spindle No. 21 was carefully immersed in a thermosensitive in-situ gel formulation. To begin with, viscosity readings were taken at different rpm values while maintaining a constant temperature of 37°C. Subsequently, the temperature was gradually increased from 25°C to 40°C while maintaining a rotational speed of 50 rpm. To evaluate the flow behaviour and temperature-dependent gelation properties of the formulation, the viscosity values under these conditions were recorded.

Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) is used to examine the surface morphology and structural characteristics of nano in-situ gel systems. By using this technique, high-resolution images can be obtained that demonstrate the distribution of nanoparticles within the polymeric gel matrix, as well as the surface texture and porous structure of the gel. Scanning Electron Microscopy (SEM) was used to evaluate the surface morphology and structural characteristics of lyophilised Levofloxacin-loaded nanoparticles. Nanoparticle powder was carefully mounted on double-sided adhesive carbon tape fixed to an aluminium stub using a small quantity of the dried powder. A thin layer of gold was applied to the sample using a sputter coater to enhance electrical conductivity and obtain clear images. After the samples had been prepared, they were analysed under appropriate vacuum conditions using a Scanning Electron Microscope (JEOL JSM-7610F, JEOL Ltd., Tokyo, Japan). We recorded micrographs under different magnifications to evaluate particle shape, surface texture, and distribution of nanoparticles. We used the obtained images to confirm the morphology and nanoscale characteristics of the formulation.

XRD STUDY(21,33)

X-ray diffraction (XRD) analysis is used to determine the crystalline or amorphous nature of the drug, polymer, and the final nanoparticle-loaded in-situ gel formulation. As a result of the interaction between the X-rays and the internal structure of the material, a diffraction pattern is produced when the rays are directed onto the sample and measured. Diffraction peaks provide information about crystal structure, degree of crystallinity, and possible interactions between the drug and excipients. The nano in-situ gel formulation was analysed using an X-ray diffractometer. Several samples, including pure drugs, polymers, and nanoparticle-loaded gel formulations, were carefully dried and placed on the sample holder. A constant scanning rate of approximately 5°–80° was utilised to record the diffraction patterns using Cu-Kα radiation as the X-ray source. To obtain clear and reproducible diffraction patterns, the instrument was operated under standard conditions.

In Vitro Drug Release Study of Nano In-situ Gel Base

A dialysis membrane diffusion method was used to assess the in-vitro drug release from the drug-loaded in-situ ophthalmic gel. An in-situ gel formulation containing nanoparticles was carefully introduced into a dialysis membrane in a volume of 5 mL. In a similar manner, 5 mL of a conventional marketed formulation was filled into another dialysis membrane for comparison. In order to prevent leakage of formulations, the open ends of the membranes were

tightly secured with a thread. As a dissolution medium, each sealed dialysis membrane was immersed separately in 100 mL of simulated tear fluid (STF) at pH 7.4. In order to maintain uniform drug distribution in the release medium, the beakers were placed in a water bath with a temperature-controlled magnetic stirrer. During specific time intervals (2, 4, 6, 8, and 12 hours), 5 mL of the release medium was withdrawn from each beaker for analysis. The sink conditions were maintained throughout the study by adding an equal volume of fresh simulated tear fluid after each sampling. The collected samples were analysed using a UV-visible spectrophotometer at the appropriate wavelength to determine the drug concentration. To study and compare the release characteristics of the formulation, the cumulative percentage of drug released was calculated and plotted against time.

***Ex-Vivo* Release Study of Nano In-Situ Gel Base**

By using a Franz diffusion cell with an excised goat cornea collected from a local slaughterhouse and positioned between the donor and recipient compartments, the nano in-situ gel was evaluated for ex vivo permeation. A simulated tear fluid of pH 7.4 was placed in the receptor compartment, maintained at 37 °C and stirred continuously with a magnetic bead to simulate ocular conditions. In the donor compartment, 1 ml of drug-loaded in-situ ophthalmic gel was placed. For the purpose of preventing evaporation, the donor compartment was left open to the atmosphere and covered. To maintain sink conditions, aliquots of 5 ml of fresh, pre-warmed receiver medium were withdrawn from the receiver compartment at predetermined intervals (15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours). Samples were filtered, if necessary, and their drug content was determined by a UV-visible spectrophotometer at a predetermined 287 λmax. According to the calibration curve of the drug, the cumulative percentage of drug permeation was calculated. A three-fold replication of the experiment was conducted to ensure reproducibility. For the purposes of comparison, a similar procedure was followed for the marketed formulation.

RESULTS

Organoleptic characterization and solubility studies

The organoleptic and solubility properties of Levofloxacin hemihydrate were evaluated before formulation development. As a pale-yellow crystalline powder, the drug was found to be odourless. In solubility studies, the drug was found to be moderately soluble in aqueous media but comparatively more soluble in ethanol. Additionally, the drug showed satisfactory solubility in simulated tear fluid and phosphate buffer solutions, indicating its suitability for use in ophthalmic drug delivery systems. For effective ocular absorption and therapeutic effectiveness, adequate dissolution of the drug is essential.

UV-vis spectrophotometric analysis

To identify the characteristic wavelength of Levofloxacin hemihydrate, the UV-visible absorption spectrum was analysed. A maximum absorption peak was observed at 288 nm, which is attributed to the electronic transitions in the quinolone ring present in the drug's molecular structure. An increase in drug concentration resulted in a corresponding increase in absorbance values, while the position of the λmax remained constant, indicating stable spectral behaviours without interference. To obtain an accurate measurement, a calibration curve was developed by plotting absorbance versus concentration in distilled water. Within the selected concentration range, the resulting plot demonstrated a strong linear relationship with the regression equation $y = 0.0064x + 0.0187$ and a correlation coefficient ($R^2 = 0.9998$). According to Beer-Lambert's law, the high linearity indicates good compliance. Based on these findings, the developed UV-visible spectrophotometric method is accurate, reliable, and appropriate for the determination of Levofloxacin in nanoparticles and in situ gel formulations. The UV spectrophotometric graph and calibration curve of the drug are shown in fig. 1.

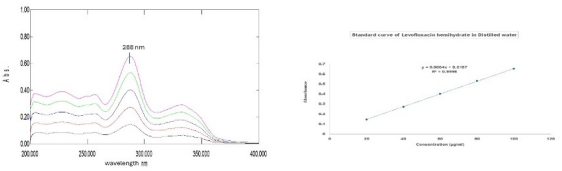

Fig. 1: UV-spectrophotometry containing (a) the UV spectrum of Levofloxacin hemihydrate and (b) the linearity curve of Levofloxacin hemihydrate

Drug- Excipient Compatibility Studies

FTIR Study

FT-IR spectra of Levofloxacin hemihydrate and its physical mixture with formulation excipients were analyzed to assess the presence of characteristic functional groups and drug–excipient compatibility. In the spectrum, a broad absorption band was observed between 3400 and 3300 cm^{-1}, which is associated with O–H stretching vibrations in the hemihydrate form of the drug as well as possible hydrogen bonding. A peak

around 2950–2850 cm^{-1} has been attributed to vibrations associated with aliphatic C–H bonds. A strong absorption band in the region of 1720–1700 cm^{-1} corresponds to the quinolone structure of Levofloxacin, while bands around 1620–1600 cm^{-1} correspond to aromatic C=C stretching and quinolone ring vibrations. There were additional peaks in the 1400–1300 cm^{-1} region that corresponded to C–N stretching, whereas there were also peaks in the 1200–1100 cm^{-1} region that corresponded to C–O stretching vibrations. The signals observed within 1000–800 cm^{-1} can be attributed to vibrations caused by aromatic C–H bending. This characteristic peak of Levofloxacin was preserved in the physical mixture without any significant shift, disappearance, or appearance of new peaks. As a result, there is no chemical interaction between the drug and the excipients, confirming the compatibility and stability of Levofloxacin within the nano in-situ gel formulation.

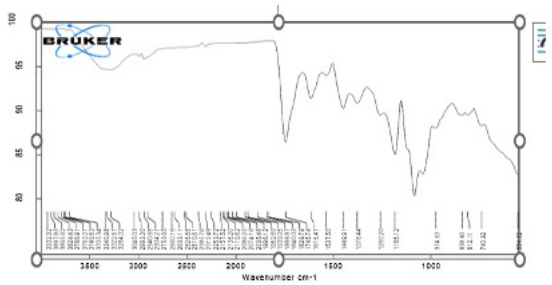

Fig. 2: FT-IR spectrum of (a) pure drug (Levofloxacin hemihydrate) and (b) drug–excipient physical mixture. The characteristic peaks of the API were retained in the physical mixture without disappearance or significant shift, indicating good compatibility between Levofloxacin hemihydrate and the selected excipients used in the formulation.

Table 2: FT-IR Spectral Peaks of the API and Their Structural Interpretations

Observed Peak (cm^{-1})	±Tolerance	Functional group assignment	Interpretation
3400-3300 cm^{-1}	±15	O–H stretching	Presence of hydroxyl groups, possibly associated with hydrogen bonding.
2950–2850 cm^{-1}	±15	C–H stretching	It is attributed to methyl and methylene groups present in the molecular structure.
1720–1700 cm^{-1}	±15	carbonyl (C=O)	It is characteristic of ketone, carboxylic acid, or ester functional groups in the compound.
1620–1600 cm^{-1}	±15	C=C stretching	It indicates the presence of aromatic or conjugated double bond systems.
1400–1300 cm^{-1}	±15	C–N stretching	It suggests the presence of amine or heterocyclic nitrogen-containing groups.
1200–1100 cm^{-1}	±15	C–O stretching	It is commonly associated with alcohols, ethers, or ester functionalities.
1000–800 cm^{-1}	±15	C–H bending	It is typically observed in aromatic or substituted hydrocarbon structures.

DSC Study

DSC data for Levofloxacin API and physical mixtures demonstrate endothermic peaks corresponding to thermal transitions of the drug and its interaction with the formulation matrix. Levofloxacin API exhibits a sharp endothermic peak at 228.4 °C, with an onset temperature of 225 °C and an endset temperature of 232.3 °C, and an enthalpy change of −41.45 mJ. Levofloxacin has a melting point that corresponds to its crystalline nature, confirming its crystalline nature and thermal stability. There is a narrow temperature

range between the onset and endset of the melting process, indicating a well-defined melting process and a high level of API purity. On the other hand, the physical mixtures containing API show an endothermic peak at 224.6 °C with an onset at 220 °C and an endset at 225.6 °C, along with a slightly higher enthalpy value (−48.63 mJ). The slight shift of the melting peak to a lower temperature compared with the pure drug suggests interaction between levofloxacin and excipients present in the formulation. This shift may occur due to partial reduction in crystallinity, molecular dispersion of the drug within the polymer matrix, or formation of weak intermolecular interactions such as hydrogen bonding between the drug and excipients. The broader onset range also indicates changes in the thermal behaviour caused by the formulation process.

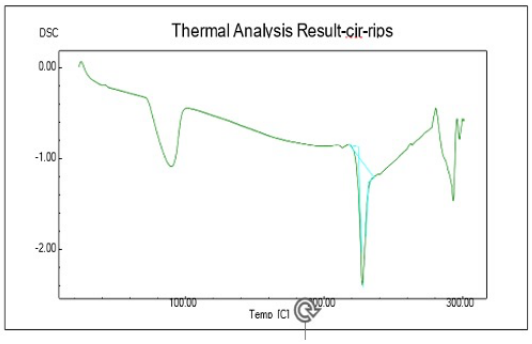

Fig 3. DSC of (a) pure drug (Levofloxacin hemihydrate) and (b) drug–excipient physical mixture.

Particle Size

All the nanoparticle formulations were evaluated, but F3 was deemed to be the optimal formulation because of its smaller particle size (5.62 nm). Nanoparticle-based drug delivery systems benefit from a smaller particle size because they increase surface area available for drug release, thereby improving dissolution rates and bioavailability. Additionally, smaller nanoparticles are often associated with better dispersion, increased permeability, and a greater ability to interact with biological membranes. Because F3 showed the lowest particle size among F1, F2, F4, F5, and F6, it was determined to be the most suitable formulation for further investigation.

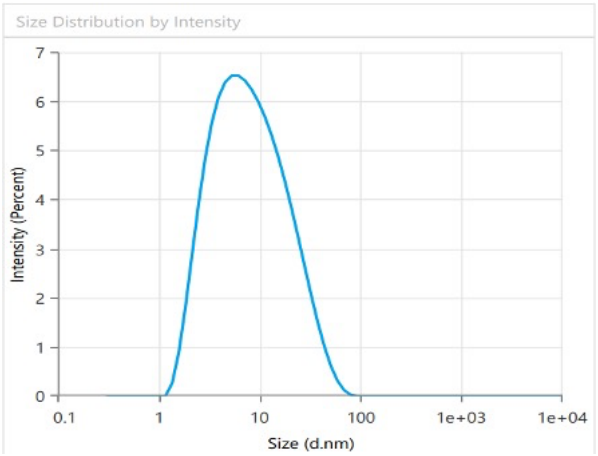

Fig 4. Particle size determinations of F3 formulations

Zeta Potential

The zeta potential is an important parameter that indicates the surface charge and stability of nanoparticle formulations. An increasing absolute zeta potential (positive or negative) results in a stronger electrostatic repulsion between particles, which prevents aggregation and improves the colloidal stability of nanoparticle systems. As a result of the preparation of these formulations, the formulation F3 demonstrated a zeta potential of −18 mV, which indicates a sufficiently high negative surface charge that can contribute to good particle stability and dispersion. The formulations F1 (4 mV), F2 (6 mV), F4 (11 mV), F5 (8 mV) and F6 (10 mV) showed relatively low zeta potential values, which may result in particle aggregation and reduced stability due to weaker electrostatic forces. As a result, F3 can be considered the optimal formulation, as it exhibits a suitable negative zeta potential that supports the stability of nanoparticles. With its small particle size and low polydispersity index, F3 exhibits better physicochemical properties than the other formulations, making it an ideal nanoparticle formulation for further research and development.

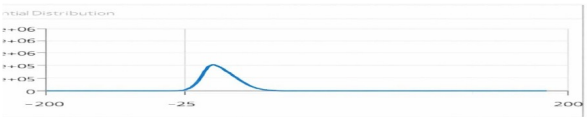

Fig 5. Zeta Potential of F3 formulations

Drug Loading and Encapsulation Efficiency

Drug loading and encapsulation efficiency are key parameters in nanoparticle drug delivery systems since they indicate the carrier system's ability to effectively incorporate and retain the drug within the nanoparticle matrix. A higher encapsulation efficiency results in a greater amount of drug being successfully entrapped within the nanoparticles, which improves

therapeutic effectiveness, reduces drug loss, and improves controlled drug release. The F3 formulation exhibited the highest drug loading/encapsulation efficiency of 96.265%, which is significantly greater than the other formulations (F1–F6). The high value indicates that almost all the drug used during formulation was incorporated into the nanoparticles. As a result of such high encapsulation efficiency, the drug and polymer/lipid matrix are in close interaction, resulting in efficient drug entrapment and reduced leakage during preparation. F1 (70.311%) and F5 (60.327%) showed a comparatively lower encapsulation efficiency, indicating a less effective drug incorporation and possible loss of drug during the formulation process. F3 was recommended as the optimal formulation because it demonstrated maximum drug loading and encapsulation efficiency, allowing for improved drug retention, improved stability of the nanoparticle system, and enhanced potential for sustained and efficient drug delivery.

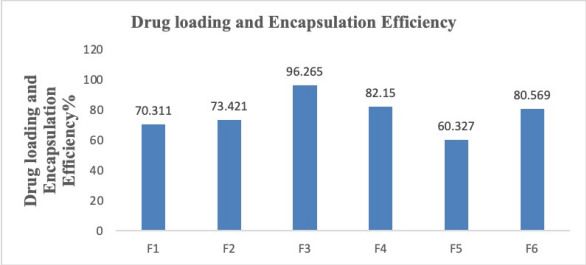

Fig 6. Percentage of Drug loading and Encapsulation efficiency

Microscopic studies of Nanoparticle

To observe the preliminary morphology and distribution of the synthesized nanoparticles, a light microscope was used to carry out a microscopic analysis of the synthesized nanoparticles. The microscopic images revealed the presence of fine particulate structures, appearing as dark granular spots dispersed throughout the medium, clearly indicating the presence of fine particulate structures. It can be concluded that the particles were relatively uniformly distributed, although some slight aggregation could be seen in some regions because of particles interacting with each other. These particulate structures are indicative of the successful synthesis of nanoparticles since they show the presence of these components.

Fig 7. Microscopic image of Nanoparticles (40x)

pH Measurement

An ophthalmic gel formulation (F1-F6) was tested for compatibility with the ocular environment using a calibrated digital pH meter. The pH values of all formulations were within the physiological range acceptable for eye preparations. A pH of 7.25 was found to be the most suitable for formulation F3, which is within the normal tear fluid range (6.8-7.3), indicating good compatibility with the eye and minimal risk of irritation. Maintaining pH stability was made possible by the appropriate concentration of Poloxamer 407 and other components. In simulated tear fluid, the formulation also retained a pH close to that of physiological tears, indicating that it is suitable for use in the eye. The pH differences in other formulations were likely caused by variations in the polymer and excipient concentrations. According to our study, F3 was identified as the ideal formulation due to its pH, which closely matches that of natural tears, thereby supporting better patient tolerance and comfort.

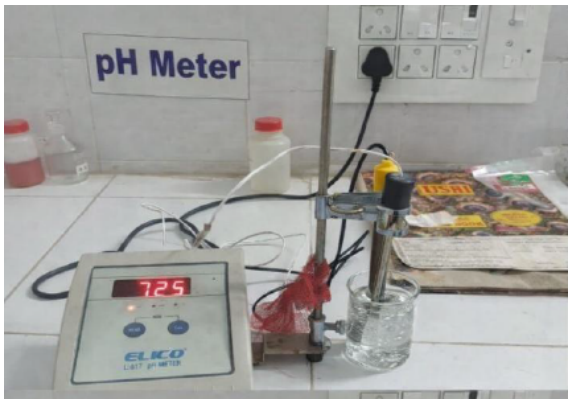

Fig 8. pH measurement of Optimised F3 formulations

Determination of the gelling capacity of the Nano In-situ gel base

By observing the sol–gel transition at physiological temperature (37 °C), the gelling capacity of the nano in-situ gel formulations (F1-F6) was determined. Among all formulations, F3 displayed the fastest

gelation time (35 s) and longest gel stability (3 h). These results indicate that F3 is more thermosensitive than the other formulations. The rapid gel formation and prolonged gel integrity may be due to the optimal concentration of Poloxamer 407, which enhances micellar aggregation and gel network formation at ocular temperatures. Accordingly, F3 was determined to be the best formulation, as it showed strong and stable gel formation compared to all other formulations.

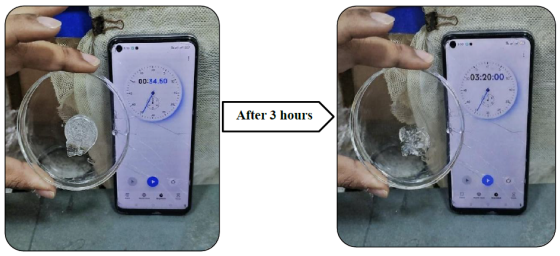

Fig 9. Gelation time of F3 formulations

Table 3. Evaluation of Gelling Capacity of Nano In-Situ Gel Formulations (F1–F6)

Formulation	Gelation time	Gel stability	Observations
F1	60s	1.5h	Moderate gel formation
F2	48s	2.0h	Good gel formation
F3	35s	3.0h	Strong, rapid and stable gel formation
F4	52s	2.1h	Moderate gel formation
F5	55s	1.8h	Weak gel formation
F6	58s	1.7h	Slightly weak gel formation

Determination of the gelling temperature of the Nano In-situ gel base

The tube inversion method was used to determine the gelling temperature of thermosensitive nano in-situ gel formulations (F1-F6). It was found that the gelling temperatures of the formulations ranged from 34.8 ± 0.20 °C to 39.2 ± 0.30 °C. F3 exhibited the optimal gelling temperature of 37.0 ± 0.15 °C, which is very close to the physiological ocular surface temperature. It was easy to instill the formulation at room temperature (25°C), and it underwent a rapid sol-gel transition when exposed to the ocular environment. The gelling temperatures for F1 and F2 were lower, which may result in premature gelation, while those for F4 to F6 were higher, which may delay the formation of the gel. Due to its ideal gelation temperature and thermosensitive behavior, F3 was considered the optimal formulation for ophthalmic in-situ gel delivery.

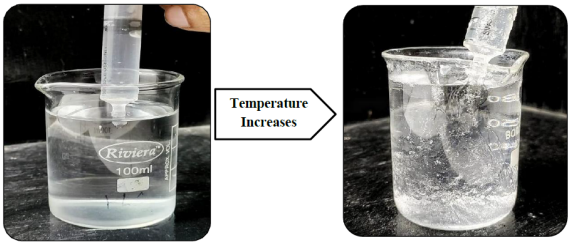

Fig 10. Determination of the gelling temperature of the Nano In-situ gel base by the tube inversion method

Determination of the Isotonicity of the Nano In-situ gel base

The isotonicity test showed that the nano in-situ gel base was isotonic with physiological fluids, as no hemolysis or morphological changes in red blood cells were observed. This indicates that the formulation is suitable and safe for ophthalmic administration.

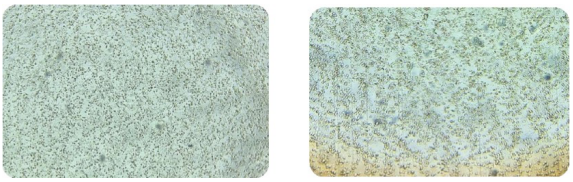

Fig 11. (a) Illustrates untreated pure RBC (b) Illustrates treated RBC with nano *In-Situ* gel

The isotonicity study revealed that the poloxamer-based nano *in-situ* ophthalmic gel formulation was isotonic with blood. Microscopic examination of RBCs treated with the formulation showed no signs of hemolysis, crenation, swelling, or morphological alteration when compared with untreated RBCs. The red blood cells maintained their normal biconcave structure and cellular integrity, indicating that the formulation did not produce any osmotic stress on the cells. Therefore, the developed nano in-situ gel formulation was confirmed to be isotonic and suitable for ophthalmic administration.

Determination of Viscosity of the Nano In-situ gel

To evaluate their thermosensitive gelation behavior, formulations F1–F6 were studied at temperatures ranging from 25°C to 37°C. The viscosity of all formulations increased with increasing temperature, indicating that the Poloxamer-based in-situ gel system

has temperature-dependent gelation properties. At 25°C, viscosity values were low (40–110 mPa·s), allowing easy administration as a liquid. Upon increasing the temperature to 37°C, increases in viscosity were observed at F1–F6, which indicated a sol–gel transition. As compared to the other formulations, F3 displayed the most suitable viscosity profile, increasing from 70 mPas at 25°C to 400 mPas at 37°C. Obtaining this balance ensures easy installation of the drug and effective gel formation in the eye, resulting in improved drug retention and sustained release. However, F1 and F2 showed lower viscosities, while F4 and F6 were excessively viscous, which might cause discomfort. As a result, F3 was identified as the optimal formulation for ocular drug delivery.

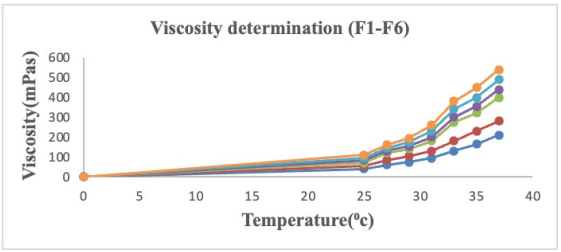

Fig 12. Viscosity determination of Nano In-situ gel (F1-F6)

The formulations F1 and F2 demonstrated low viscosities, which may result in weaker gel formation and reduced ocular residence time. F3 showed a balanced viscosity profile, remaining sufficiently fluid at room temperature for easy instillation while forming a strong gel at ocular temperature, which contributes to enhanced drug retention and sustained release. However, F4, F5, and F6 have very high viscosities at elevated temperatures, which may cause difficulty during instillation and may cause blurred vision or discomfort to the patient.

Scanning Electron Microscope study

Scanning electron microscopy was used to examine the surface morphology of nano in-situ gel formulations (F1, F3, and F6). The SEM images showed noticeable variations in particle morphology and scaffold structure among the formulations. Formulation F1 showed spherical particles with minor surface irregularities such as small pits and cracks, indicating moderate structural stability due to possible polymer shrinkage during drying. F3 exhibited well-defined spherical particles with a smooth and uniform surface, suggesting efficient nano in-situ gel formation and homogeneous polymer–drug distribution. In contrast, F6 displayed a heterogeneous scaffold with fragmented particles, porous debris, and partially collapsed structures, indicating weaker gel network formation. Among the formulations, F3 showed the most desirable morphology, characterised by superior sphericity, smooth surface, and better structural integrity, suggesting improved stability and potential for sustained drug release.

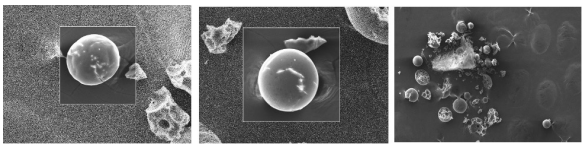

Fig 13. (a) Scaffold image of (F1) formulations, (b) Scaffold image of (F3) formulations and (c) Scaffold image of (F6) formulations

XRD Analysis of Levofloxacin-Loaded Nano In-Situ Gel

An X-ray diffraction (XRD) analysis was conducted to evaluate the crystallinity of pure levofloxacin (API) and the optimised F3 nano in-situ gel formulation. The diffraction peaks of the pure drug were sharp and intense at specific 2θ values, confirming its highly crystalline nature. The F3 formulation, on the other hand, displayed reduced peak intensities and broader diffraction patterns, indicating a decrease in crystallinity and a partial conversion into an amorphous or molecularly dispersed state within a polymeric gel matrix. This indicates that levofloxacin has been successfully incorporated into the nano in-situ gel system and has been dispersed uniformly. As a result of the reduced crystallinity of F3, the drug may be more solubilised, dissolved, and bioavailable. As well, the absence of new peaks indicates that there is no chemical interaction between the drug and polymers, thereby confirming compatibility between the drug and polymers. Therefore, F3 was the best formulation due to its improved dispersibility and stability within the nano in-situ gel matrix.

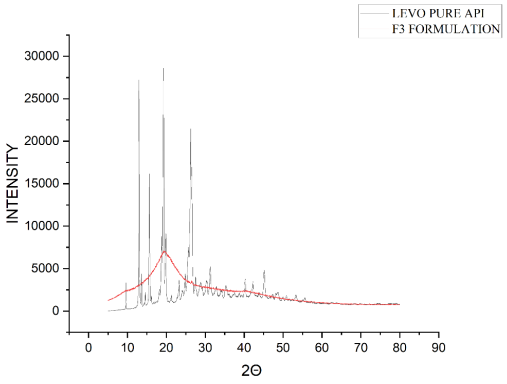

Fig 14. XRD study of Levofloxacin-loaded Nano In-situ gel

In-Vitro Drug Release Study

The in-vitro dissolution study aimed to compare the drug release behaviour of the developed thermosensitive nano in-situ gel formulations (F1-F6) with the marketed formulation. As a result, there was a significant difference between the release profiles of the marketed product and those of the prepared formulations. Based on the results of all formulations, F3 showed the most desirable release profile, with a cumulative drug release rate of approximately 90% at 6 hours and a gradual release pattern throughout the study period. The improved performance of F3 may be attributed to the optimal polymer concentration and formation of a uniform gel network, which facilitated drug entrapment and controlled drug diffusion more effectively. The formulations F1 and F2 showed relatively slower drug release due to low polymer interactions within the gel matrix. The F4, F5, and F6 gels released drugs relatively faster, possibly due to weaker gel strength or higher porosity. It was determined that F3 was the most effective formulation based on the results of dissolution, as it demonstrated a balanced and sustained release of the drug, improved gel stability, and better control of drug diffusion in comparison with the other formulations and the marketed product.

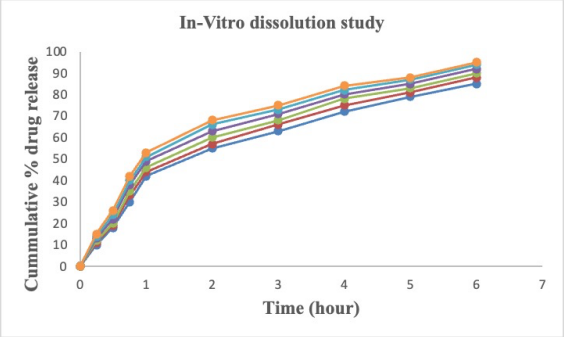

Fig 15. In-Vitro dissolution study Nano In-situ gel (F1-F6)

Ex-Vivo Study of Nano In-situ gel base

During the ex-vivo diffusion study of nano in-situ gel formulations (F1-F6), the drug gradually permeated across the biological membrane over a period of six hours. It was observed that all formulations exhibited sustained release behaviour; however, variations in diffusion rates were observed. Among the formulations, F3 showed the best release profile, achieving 87% cumulative drug release at 6 hours with steady and controlled distribution. The improved performance of F3 may be attributed to the optimal polymer concentration and formation of a stable gel network, which facilitated controlled drug permeation. As a result of differences in gel strength and matrix porosity, Formulas F1 and F2 displayed slower diffusion, whereas Formulas F4, F5, and F6 displayed faster release. The optimal formulation was therefore considered to be F3 due to its balanced ex vivo drug diffusion and sustained release characteristics.

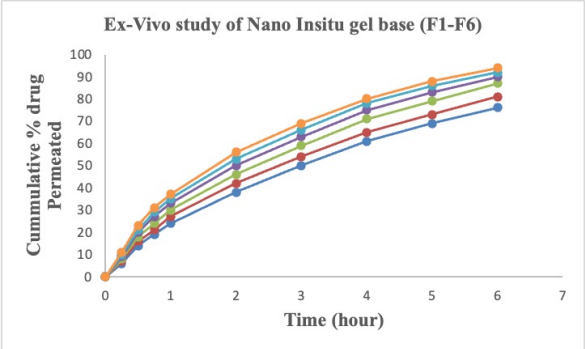

Fig 16. Ex-Vivo study data of Nano In-situ gel base (F1-F6)

DISCUSSIONS:

Our study successfully developed levofloxacin-loaded polymeric nanoparticles integrated in a thermosensitive in-situ gel system for ocular drug delivery. The formulation approach was designed to enhance precorneal retention, enhance drug entrapment, and provide sustained drug release over conventional ophthalmic preparations. Out of the six formulations evaluated, F3 consistently demonstrated superior performance in terms of physicochemical properties, gelation behaviour, surface morphology, and drug release profile.

Levofloxacin hemihydrate was found to be suitable for the development of ocular formulations after the preformulation studies. There was acceptable solubility of the drug in aqueous media, simulated tear fluid, and phosphate buffer, indicating its potential for use in the treatment of eye diseases. The UV spectrophotometric method showed excellent linearity at 288 nm, confirming its validity for drug estimation during formulation and release studies.

Compatibility studies using FT-IR and DSC showed that levofloxacin was compatible with the selected excipients. The FT-IR peaks characteristic of the drug were retained in the physical mixture without any significant shift or disappearance, suggesting no chemical incompatibility. The DSC analysis also showed only a slight shift in the melting endotherm of the drug in the physical mixture, which could be attributed to reduced crystallinity or molecular dispersion of the drug in the polymer matrix rather than chemical interactions. Based on these findings, it

can be concluded that the selected polymers and excipients are suitable for the formulation of nanoparticles and in-situ gels.

Nanoparticle performance is influenced by particle size and zeta potential. Among the formulations tested, F3 exhibited the smallest particle size and a relatively high negative zeta potential, which indicates greater colloidal stability and reduced aggregation tendency. The smaller particle size may result in greater surface area, dissolution, and contact with biological membranes, whereas the negative zeta potential supports the physical stability of the nanoparticulate system. Additionally, F3 demonstrated the highest level of encapsulation efficiency, suggesting that levofloxacin is effectively encapsulated within the polymeric matrix. As a result of optimizing the concentration of PVA and PLGA, the emulsion stability and drug retention were likely to be improved during the preparation of nanoparticles.

All formulations were found to have pH values within acceptable ranges for ophthalmic use, but formulation F3 was found to be the most suitable pH value, closely matching the tear pH. Ocular compatibility, patient comfort, and a reduction of irritation are important factors to consider when administering a drug. During the isotonicity study, no significant changes to red blood cell morphology were observed. This further confirms the safety of the developed formulation. The results of this study support the suitability of in-situ gels for use in ophthalmic applications.

A crucial aspect of the performance of the in-situ gel developed was its thermosensitive nature. In terms of gelation characteristics, F3 had the most desirable characteristics, including a rapid gelation time, a prolonged gel stability, and a gelation temperature that was close to the surface temperature of the eye. The findings suggest that the formulation can be administered as a liquid and rapidly converted into a gel after administration, resulting in a prolonged residence time in the eye. As a result of viscosity studies, F3 was also found to remain sufficiently fluid at room temperature, however it showed an increase in viscosity at 37 degrees Celsius. Ophthalmic in-situ gels should behave in such a manner in order to facilitate both administration and prolonged contact with the ocular tissues.

A morphological analysis of selected formulations using SEM revealed clear differences in morphology. The F1 scaffold consisted of spherical particles with minor surface imperfections, while the F6 scaffold showed fragmented structures and a disrupted and heterogeneous scaffold. F3 exhibited a smooth, spherical, uniform morphology with improved structural integrity. As a result, nanoparticles were formed and distributed homogeneously within the gel matrix, which may result in enhanced stability and controlled drug release. XRD analysis further demonstrated F3's optimized nature by demonstrating reduced crystallinity of levofloxacin. As a result of the reduction in peak intensity and broadening of diffraction peaks, the drug may be partially converted into amorphous or molecularly dispersed form, which may improve its solubility and dissolution properties.

The in-vitro drug release study demonstrated that the developed nano in-situ gel formulations provided sustained release compared to the marketed formulation. Among all formulations, F3 exhibited the most balanced and desirable release profile, resulting in gradual drug release over a period of 6 hours. This sustained release behavior may be due to a combination of nanoparticle entrapment and thermosensitive gel matrix formation, both of which reduce the rate of drug diffusion. F1 and F2 displayed slower release, possibly due to tighter entrapment or a less optimized gel structure, whereas F4, F5, and F6 displayed faster release, which may be related to higher porosity or a weaker gel network. Accordingly, F3 provided the best balance between drug retention and controlled release.

The ex-vivo permeation study conducted on excised goat cornea further confirmed F3's suitability as the optimal formulation. Over the course of 6 hours, F3 showed steady and sustained drug permeation, which suggests that the formulation was capable of maintaining prolonged drug availability at the ocular surface and allowing controlled permeation across the corneal membrane. For ocular drug delivery, this behavior is highly desirable due to its potential to reduce dosing frequency and improve therapeutic efficacy.

Based on the results obtained, it appears that the optimized F3 formulation successfully combines the advantages of both polymeric nanoparticles and in-situ gel systems. As a result of the formulation, favorable physicochemical properties were demonstrated, compatibility was established, gelation behavior was appropriate, ocular tolerance was improved, morphology was improved, crystallinity was reduced, and both in vitro and ex vivo drug release was sustained. According to these findings, levofloxacin-loaded nanoparticles in-situ gel may be

an effective ophthalmic delivery system for the sustained and targeted treatment of bacterial infections.

CONCLUSION:

The present study developed and evaluated polymeric nanoparticles containing levofloxacin, incorporated into a thermosensitive in-situ gelling system for sustained ocular drug delivery. Through the use of PLGA and Eudragit RL100 polymers, nanoparticles were prepared through the oil-in-water emulsification solvent evaporation method and further incorporated into a poloxamer-based in situ gel. The formulation F3 demonstrated the most desirable physicochemical and pharmaceutical properties among the developed formulations (F1-F6). The particle size of the sample was the smallest, the zeta potential was optimal (18 mV), and the encapsulation efficiency was the highest (96.265%), indicating good nanoparticle stability and effective drug encapsulation. The optimised formulation also demonstrated acceptable ocular pH, rapid gelation time, and an ideal gelation temperature close to physiological ocular temperature. SEM analysis confirmed smooth, spherical nanoparticles, whereas XRD studies showed reduced crystallinity of levofloxacin within the polymeric matrix, indicating improved dispersion. Moreover, in vitro and ex vivo studies have demonstrated sustained drug release and enhanced permeation. Overall, the optimised nano in-situ gel system shows promising potential for the delivery of ophthalmic drugs for sustained treatment of bacterial eye infections.

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