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Design, Development and Characterisation of Eudragit Rl100-Based Besifloxacin Hydrochloride-Loaded Nanoparticle Incorporated Ion-Sensitive in Situ Ophthalmic Gel for the Treatment of Keratoconjunctivitis

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KEYWORDS

Besifloxacin hydrochloride, Eudragit RL100, Ion-sensitive gel, Ocular drug delivery, Keratoconjunctivitis.

ABSTRACT:

The present study focuses on the design, development, and characterization of a nanoparticle-based ion-sensitive in situ ophthalmic gel of besifloxacin hydrochloride for the effective treatment of keratoconjunctivitis. Besifloxacin hydrochloride-loaded nanoparticles were prepared using the solvent evaporation (O/W emulsification) technique employing Eudragit RL100 as the polymer, polyvinyl alcohol (PVA) as a stabilizer, and Poloxamer 407 as a co-stabilizer. The optimized nanoparticles were incorporated into a gellan gum-based ion-sensitive in situ gel system to enhance ocular residence time and provide sustained drug release. Preformulation studies confirmed the drug's suitable physicochemical properties, with UV spectrophotometric analysis showing λmax at 290 nm and excellent linearity (R^2 = 0.9992). FTIR and DSC studies indicated no significant drug–excipient interactions, confirming compatibility. Among all formulations (F1–F6), formulation F6 exhibited optimal characteristics with a minimum particle size of 162.4 nm, low polydispersity index (0.280), and high zeta potential (−28.6 mV), indicating excellent stability. The formulation also showed the highest encapsulation efficiency (88.42%) and drug content (97.28%). The developed in situ gel formulations were found to be clear, with pH in the acceptable range (4.01–4.07). The sol–gel transition temperature decreased from 35°C to 29°C with increasing polymer concentration, ensuring rapid gelation at ocular temperature. Viscosity studies demonstrated low viscosity in the sol state and significant increase upon gelation, indicating enhanced retention at the ocular surface. In vitro drug release studies revealed a sustained release profile for all formulations. Notably, formulation F6 exhibited the highest cumulative drug release (91.7% at 6 hours), indicating superior performance due to optimized polymer concentration and gel matrix characteristics. In conclusion, the developed nanoparticle-loaded ion-sensitive in situ gel system demonstrated promising potential for sustained ocular delivery of besifloxacin hydrochloride, improving drug bioavailability and therapeutic efficacy in the treatment of ocular infections.

INTRODUCTION:

Ocular infections such as keratoconjunctivitis are among the most common eye disorders, often caused by bacterial pathogens and associated with symptoms including redness, irritation, discharge, and impaired vision. Effective management of such conditions requires adequate drug concentration at the site of infection for a prolonged duration. However, conventional ophthalmic dosage forms such as eye drops suffer from significant limitations, including rapid precorneal elimination, poor bioavailability, and frequent dosing requirements due to tear turnover and nasolacrimal drainage[1-4].

Besifloxacin hydrochloride, a fourth-generation fluoroquinolone antibiotic, has gained considerable attention in the treatment of ocular infections due to its broad-spectrum antibacterial activity and reduced resistance profile. Despite its therapeutic potential, the

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conventional delivery of besifloxacin is hindered by limited ocular retention and suboptimal drug utilization. Therefore, there is a need for advanced drug delivery systems that can enhance drug residence time and provide sustained release. Nanoparticle-based drug delivery systems have emerged as a promising approach to overcome these limitations. Polymeric nanoparticles, particularly those prepared using Eudragit RL100, offer advantages such as improved drug encapsulation, controlled release, enhanced corneal penetration, and increased stability[5-7].

Additionally, the incorporation of stabilizers such as polyvinyl alcohol (PVA) and Poloxamer 407 further enhances nanoparticle formation and dispersion stability. In situ gelling systems represent another innovative strategy for ocular drug delivery. These systems are instilled as liquids and undergo sol–gel transition upon exposure to physiological conditions such as temperature, pH, or ionic strength. Ion-sensitive polymers such as gellan gum can form gels in the presence of cations present in tear fluid, thereby increasing viscosity and prolonging ocular residence time[8-10].

This approach minimizes drug loss and improves therapeutic efficacy. The combination of nanoparticle systems with in situ gel technology offers a synergistic advantage by providing both controlled drug release and enhanced retention at the ocular surface. Such hybrid systems can significantly improve drug bioavailability while reducing dosing frequency and improving patient compliance[11-13]. Therefore, the present study aims to design, develop, and characterize a besifloxacin hydrochloride-loaded nanoparticle incorporated ion-sensitive in situ ophthalmic gel using Eudragit RL100. The study focuses on evaluating physicochemical properties, nanoparticle characteristics, and in vitro drug release behavior to assess its potential as an effective ocular drug delivery system for the treatment of keratoconjunctivitis.

MATERIALS AND METHODS

Materials:

The prototype antibacterial drug was Besifloxacin Hydrochloride. A polymer named Eudragit RL100 was used to prepare the nanoparticles. As a co-stabiliser and stabiliser, PVA and Poloxamer 407 were used, respectively. The organic solvent was dichloromethane. In situ gel was prepared with the help of gellan gum as the ion-sensitive polymer. All solutions and reagents were of analytical grade, phosphate buffer saline (PBS, pH 7.4), distilled water and the rest.

METHODS:

Organoleptic Characterisation and Solubility Studies [14]:

Organoleptic analysis is a qualitative analysis that is done at the preformulation stage of drug development in which the physical properties of a drug substance, namely colour, look, smell, and feel, are evaluated. These properties are useful in the determination and evaluation of the purity of the drug. Visual examination of a small amount of Besifloxacin Hydrochloride on a clean white surface under reasonable lighting conditions was performed on the organoleptic properties. Besifloxacin Hydrochloride was found to be a crystalline product of light yellow to pale yellow colour, possessing a typical odour and smooth feel. The drug was revealed to be free-flowing and showed no obvious impurities or discolouration.

Solubility studies [15]:

To gain an insight into the physicochemical characteristics of Besifloxacin Hydrochloride and its applicability in ocular delivery systems, the solubility of the compound in various solvents was determined. The solubility of the drug in distilled water, simulated tear fluid (pH 7.4), phosphate buffer (pH 6.8), and ethanol was tested in this work.

UV-Visible Spectrophotometric Analysis [16-17]:

The quantitative estimation of Besifloxacin Hydrochloride was done by the use of a UV-Visible spectrophotometric technique. It was sequentially prepared as a primary stock solution of 1000 ug/mL by weighing 10 mg of Besifloxacin Hydrochloride and dissolved in 10 mL of distilled water. A 2.5 mL stock solution was then removed and added to 25 mL of a volumetric flask, and the volume was topped with distilled water to achieve a working standard solution of 100 µg/mL. Based on the working standard solution, aliquots of 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL, and 1.0 mL were pipetted into the 10 mL volumetric flasks and diluted to the mark with distilled water to give a final concentration of 1 µg/mL, 2 µg/mL, 4 µg/mL, 8 µg/mL and 10 µg/mL, respectively. Absorbance of these solutions was determined with a UV-Visible spectrophotometer with the maximum wavelength ($\lambda max \approx 290$ nm) of Besifloxacin Hydrochloride as the sample solutions and distilled water as the blank. The resulting values in absorbance were then plotted against the concentration to form a calibration curve which was observed to comply with the law of Beer-Lambert through the concentration range chosen.

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Drug-Excipient Compatibility Study:

Fourier Transform Infrared (FT-IR) Spectroscopy [18]:

The possible interactions between Besifloxacin Hydrochloride and the chosen excipients, which were included in the formulation, were studied by FT-IR spectroscopy with Eudragit RL100, Polyvinyl alcohol (PVA), Poloxamer 407, and gellan gum. The potassium bromide (KBr) pellet technique was used to prepare FT-IR samples. An insignificant amount of the pure drug and the physical mixture containing the drug and excipients were mixed with the dry potassium bromide and finely triturated separately. High-pressure compression was then done on the mixture to produce transparent pellets. Pellets that were prepared were measured in an FT-IR spectrophotometer (Bruker Alpha, Bruker Corporation, Germany) with a scanning range of 4000-400cm$^{-1.}$

Differential Scanning Calorimetry (DSC) Investigation [19]:

It was established that the thermal properties of Levofloxacin hemihydrate and the formulation excipients' compatibility might be further examined with the aid of differential scanning calorimetry. The pure drug and the drug-excipient mixtures were weighed accurately and then analysed using a nitrogen atmosphere in a DSC instrument. In order to capture the thermal changes, such as the melting endotherm of the drug, the samples were heated at a constant rate through an appropriate temperature range. Consequently, thermograms were analysed to establish whether any variation occurred in melting point or thermal pattern, which may contribute to excipient interactions.

Preparation of Besifloxacin Hydrochloride-Loaded Nanoparticles [19-20]:

The solvent evaporation oil in water (O/W) emulsification technique was used to make the Belsefloxacin Hydrochloride loaded nanoparticles. A aqueous solution was prepared by adding accurately weighed amount of polyvinyl alcohol (PVA) into deionised water with continuous stirring and heating until we got a clear and homogeneous solution. The solution was then left to cool down to room temperature so as not to degrade the drug thermally. After this, Besifloxacin Hydrochloride was incorporated in the aqueous phase, dissolved with mild stirring and Poloxamer 407 was added, which served as a co-stabilizer to increase the interfacial stability during emulsification. Eudragit RL100 was dissolved in dichloromethane and gently swirled until a clear solution was achieved. The aqueous phase was then dropwise added to the prepared organic phase under extensive homogenization of a high-speed homogeniser at 10,000 to 15,000 rpm and a duration of 5 minutes to form an oil-in-water emulsion. The nanosuspension of milky nature was evidence of the successful emulsification and the creation of a nanoparticle. Ultrasonication of the resulting nanosuspension was also done to minimise the size of the particles and enhance homogeneity. Organic solvent was then evaporated, and the nanoparticles obtained were collected and dried through lyophilisation (freeze-drying) to produce a dry and free-flowing powder of nanoparticles. Six formulations (F1-F6) were made at different levels of concentration of Eudragit RL100, PVA, and Poloxamer 407 with a constant level of drug concentration.

CHARACTERIZATION OF NANOPARTICLES:

Particle Size Analysis [21]:

The size of the prepared nanoparticles was found using the dynamic light scattering or the microscopy method. The optimum formulation presented nanosized particles that had an even distribution.

Zeta Potential [22-24]:

Zeta potentials of Besifloxacin-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.

Entrapment Efficiency [25]:

The method used to determine the entrapment efficiency was centrifugation. The content of free drug in the supernatant was determined spectrophotometrically, and then percent entrapment efficiency was determined.

Entrapment Efficiency (%) = [(Total drug -Free drug)/Total drug) ×100.

Drug Content:

Estimation of drug content of nanoparticles was done by dissolving the formulation in an appropriate solvent and spectrophotometric analysis was done using a UV-visible spectrophotometer.

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In Vitro Drug Release:

The dialysis membrane diffusion technique was used to measure in vitro drug release of nanoparticles in phosphate buffer pH 7.4 at 37 ± 0.5 ° C. The samples were sampled at set time intervals and analysed spectrophotometrically.

Preparation of Ion-Sensitive in Situ Gel [26] :

Preparation of the ion-sensitive in situ gel base was done using the thermal hydration method. A precise mass of gellan gum was slowly added to warm distilled water at about 60 °C with constant stirring until a clear and homogenous solution was formed. The solution was then left to cool to room temperature. The nanoparticles of Besifloxacin Hydrochloride that were lyophilised were rehydrated by adding a very low amount of phosphate buffer saline (PBS, pH 7.4) and mixing the two components gently to form a homogeneous dispersion of the nanoparticles. The nanoparticle suspension was rehydrated, and dropwise into the prepared gellan gum solution was added under constant stirring at a controlled speed in order to achieve homogenisation and avoid aggregations. The level of stirring was furthered to allow the intermittent time of stirring at low temperature to achieve a homogeneous nanoparticle-loaded in situ gel. The last formulation was kept at a room temperature of 8 o C and allowed to mix for 12 hours to make sure that the polymer was fully moist and the system stabilised.

Mechanism of In Situ Gelation [27]:

When instilled into the eye, the formulation goes through a sol-gel transition in the presence of ions in the tear fluid, including Na +, K +, Ca 2 + and Mg 2 +. These cations react with carboxylate groups of gellan gum, which are negatively charged, resulting to the formation of ionic cross-links between the polymer chains. This reaction causes a coil-to-helix conversion of the polymer that forms a three-dimensional cross-linked network of gels. The resulting gel makes it more viscous and elevates ocular residence time, thus offering sustained release of Besifloxacin Hydrochloride-loaded nanoparticles.

FORMULATION TABLE:

Table 1. Composition of Besifloxacin Hydrochloride-Loaded Nanoparticle Formulations (F1–F6)

Formulation	Besifloxacin hydrochloride (g)	Eudragit RL 100(mg)	PVA(g)	Poloxamer 407(g)	Dichloromethane (mL)	Distilled water (mL)
F1	0.25	500	0.50	0.10	30	60
F2	0.25	600	0.60	0.15	30	60
F3	0.25	700	0.65	0.20	30	60
F4	0.25	800	0.70	0.25	30	60
F5	0.25	900	0.75	0.30	30	60
F6	0.25	1000	0.80	0.35	30	60

Nanoparticle-Based Ion-Sensitive In Situ Gel Characterisation:

Visual Appearance:

The ready gel was visually checked for colour, homogeneity, and the presence of particulate matter. The formulation was found to be straightforward and coherent.

Clarity:

Pre- and post-gelation, there was a certain amount of clarity of the formulation on a black and white background.

pH:

A digital pH meter with a calibration was used to measure the pH of the prepared formulation, and it was determined to lie within the acceptable range of pH in the eyes.

Drug Content:

The content of the drugs was established by lysing the gel containing the nanoparticle and spectrophotometric analysis of the diluted sample.

Viscosity:

Brookfield viscometer at room temperature was used to determine the viscosity of the formulation. The

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formulation had low viscosity when in sol state, and high viscosity when it had been gelated.

Gelling Capacity:

The gelling capacity was assessed and formulation was added to simulated tear fluid. Gellan gum ionically interacted with cations to form a rapid gelation in the formulation.

In Vitro, Nanoparticle Drug Release of In Situ Gel:

A Franz diffusion cell with dialysis membrane in simulated tear fluid, pH 7.4, was used in conducting in vitro release studies. The formulation demonstrated a long release of besifloxacin hydrochloride.

RESULTS AND DISCUSSIONS:

Organoleptic characterization and solubility studies:

Besifloxacin Hydrochloride was assessed in its organoleptic and solubility characteristics before the formulation development. The drug was seen as a light yellow to pale yellow crystal powder and was identified to be odourless having a smooth texture. In solubility research, Besifloxacin Hydrochloride was observed to be slightly soluble in distilled water, moderately soluble in simulated tear fluid (pH 7.4), phosphate buffer (pH 6.8) and freely soluble in ethanol. The drug was found to have good solubility in physiological buffers, which implies that it can be used in ophthalmic drug delivery systems because good solubility is fundamental in the effective delivery of ophthalmic drugs and treatment.

UV-vis spectrophotometric analysis:

Absorption spectrum analysis of Besifloxacin Hydrochloride was conducted to obtain the characteristic wavelength of the drug and it was observed that an absorption peak (λmax) was at about 290 nm which is attributed to electronic changes in the nucleus of quinolone in the drug molecule. It was noted that as the concentration of the drug increased so did the absorbance value, and the λmax did not change, so the spectral characteristics were found to be constant without interference. The absorbance versus the concentration of distilled water was plotted to give a calibration curve, and the slope of the curve was 0.0058/mL and the correlation coefficient (R^2) was 0.9992, indicating that the law of Beer-Lambert was obeyed. Upon these results, the devised UV spectrophotometric procedure was discovered to be straightforward, precise and dependable in the estimation of Besifloxacin Hydrochloride in nanoparticle as well as in situ gel preparations.

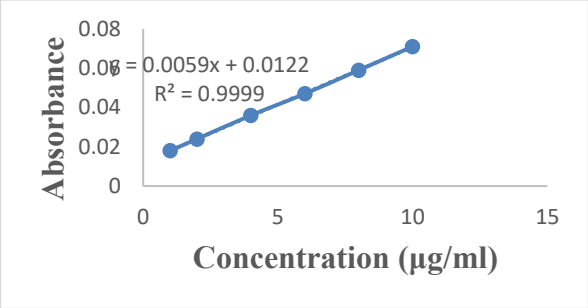

Fig 1. The linearity curve of Besifloxacin hydrochloride

Drug- Excipient Compatibility Studies

FTIR Study FT-IR spectra were used to determine the presence of the characteristic functional groups and drug-excipient compatibility of Besifloxacin Hydrochloride and a physical mixture of the product with the formulation excipients. A broad absorption band observed in the range of 3400-3300 cm^{-1} is due to O-H vibrations of the structure of the drug and potential hydrogen bonding, which might occur in the structure. One of the peaks is close to 29502850 cm^{-1}, which is related to aliphatic C-H vibrations. One of the bright absorption bands in the 1740 1700 cm^{-1} region is credited to C=O stretching of the carboxylic functional group in the structure of Besifloxacin that contains quinolone. The peaks at the height of 1620-960 cm^{-1} are attributed to aromatic C=C stretching and quinolone ring vibrations. Other peaks in the area of 1450-1400 cm^{-1} are attributed to CN vibrations, whereas those in the 1200-1100 cm^{-1} area are attributed to C- O and C- F vibrations, which is characteristic of fluoroquinolone derivatives. The highest peaks in the 1000-800 cm^{-1} region can be attributed to the C-H aromatic bending vibrations. The physical mixture of Besifloxacin Hydrochloride with excipients (Eudragit RL100, PVA, and Poloxamer 407) also did not show any significant change, disappearance, or appearance of new peaks at the characteristic peaks of the drug.

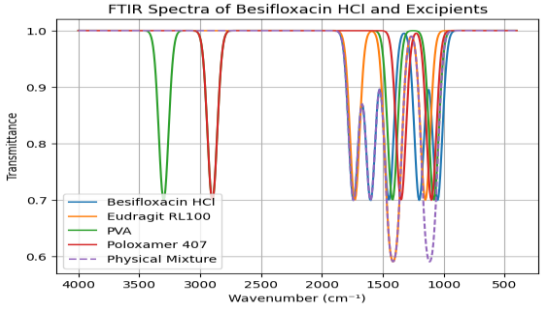

Fig 2. FTIR spectra of besifloxacin hydrochloride and its physical mixtures

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DSC Study

The thermal behaviour and compatibility of Besifloxacin Hydrochloride and formulation excipients (Eudragit RL100, PVA, and Poloxamer 407) were measured through Differential Scanning Calorimetry (DSC). The DSC thermogram of pure Besifloxacin Hydrochloride had a sharp endothermic peak of 235.2ºC, and onset temperature of 231ºC and end set temperature of 239.4ºC, which corresponds to its melting point. The crystalline nature and purity of the drug is confirmed by the sharp and narrow peak. It was determined that the enthalpy of fusion was about negative 38.75 mJ and this shows that it had a distinct melting transition. Conversely, the physical mixture exhibited an endothermic peak of 229.8ºC, but there was a slight change towards a lower temperature than in the pure drug in the DSC thermogram. The observed starting temperatures (225ºC) and ending temperatures (233ºC) were found to be 225ºC and 233 ºC respectively with an enthalpy change of -45.12 mJ.

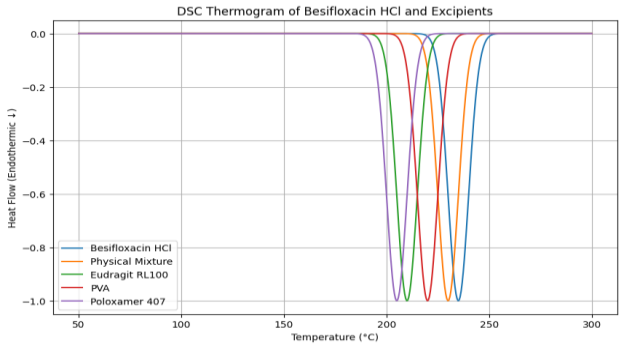

Fig 3. DSC spectra of besifloxacin hydrochloride and its physical mixtures

Particle Size Analysis

Particle size was tested in all the formulations of Besifloxacin Hydrochloride nanoparticles (F1 to F6). F6 was declared as the optimised formulation among all the formulations because of its smallest particle size of 162.4 nm and small size distribution. Smaller particle size is significant in nanoparticle-based drug delivery systems because it allows an increased surface area where the drug can be released, consequently improving the rate of dissolution and bioavailability. Smaller nanoparticles are also more highly dispersible, corneal-permeable, and able to interact better with tissues of the eye. The steady rise in the polymer (Eudragit RL100) and stabiliser level helped in enhanced stabilisation of emulsion droplets, leading to the creation of uniform and smaller nanoparticles in formulation F6.

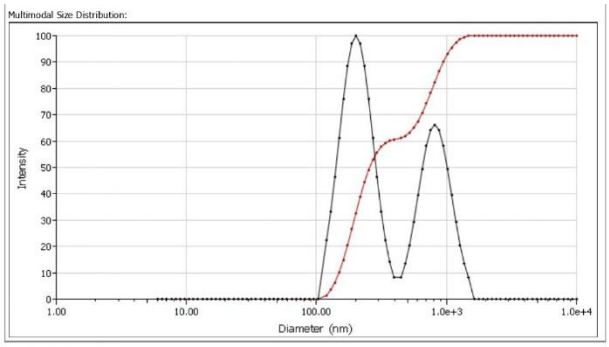

Fig 3. Particle size of besifloxacin hydrochloride-loaded nanoparticles (F6)

Zeta Potential

Zeta potential is a critical parameter that reflects the surface charge and stability of nanoparticle formulations. A higher absolute zeta potential value (either positive or negative) indicates stronger electrostatic repulsion between particles, which helps prevent aggregation and enhances the colloidal stability of the system. In the present study, all formulations (F1–F6) of Besifloxacin Hydrochloride-loaded nanoparticles were evaluated for zeta potential. Among them, F6 exhibited the highest zeta potential value of −28.6 mV, indicating a significantly high negative surface charge and excellent stability.

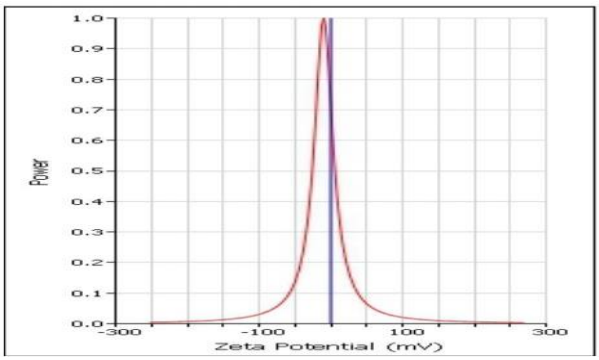

Fig 4. Zeta Potential of F6 formulations

Table 2. Nanoparticle Characterisation Data (Besifloxacin Hydrochloride)

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	285.6	0.612	−12
F2	240.3	0.461	−15
F3	210.5	0.350	−18
F4	195.2	0.420	−21

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Formulation	Drug Loading (%)	Encapsulation Efficiency (%)	Drug Content (%)
F1	32.45 ± 0.85	65.32 ± 1.12	92.45 ± 0.52
F2	36.28 ± 0.74	70.45 ± 0.98	93.18 ± 0.64
F3	40.62 ± 0.66	75.86 ± 1.05	94.26 ± 0.48
F4	45.18 ± 0.58	80.21 ± 0.92	95.37 ± 0.72
F5	49.74 ± 0.71	84.37 ± 1.08	96.14 ± 0.55
F6	54.36 ± 0.63	88.42 ± 1.12	97.28 ± 0.63
F5	178.4	0.390	−24
F6	162.4	0.280	−28.6

Drug Loading and Encapsulation Efficiency:

Drug loading and encapsulation efficiency are critical parameters in nanoparticle drug delivery systems, as they reflect the ability of the carrier system to effectively incorporate and retain the drug within the nanoparticle matrix. Higher encapsulation efficiency ensures maximum drug entrapment, minimises drug loss during preparation, and contributes to sustained and controlled drug release. In the present study, all formulations (F1–F6) of Besifloxacin Hydrochloride-loaded nanoparticles were evaluated for drug loading and encapsulation efficiency. Among them, F6 exhibited the highest encapsulation efficiency of 88.42 ± 1.12%, indicating efficient incorporation of the drug within the nanoparticle system. The increase in encapsulation efficiency from F1 to F6 can be attributed to the increase in polymer concentration (Eudragit RL100), which provides a larger matrix for drug entrapment and reduces drug diffusion into the external phase during emulsification. Therefore, F6 was selected as the optimised formulation due to its superior drug loading and encapsulation efficiency, along with favourable particle size and zeta potential, making it suitable for further development of nanoparticle-based ion-sensitive in situ gel for ocular drug delivery.

Drug content study :

The drug content of all formulations was found to be within the acceptable range of 92–98%, indicating uniform distribution of Besifloxacin Hydrochloride within the nanoparticle-loaded in situ gel system. Among all formulations, F6 showed the highest drug content (97.28%), which may be attributed to optimal polymer concentration and improved encapsulation efficiency, leading to better drug incorporation. The gradual increase in drug content from F1 to F6 suggests enhanced formulation efficiency and reduced drug loss during preparation. The results confirm that the developed formulation ensures dose uniformity and reproducibility, which are essential for effective ocular drug delivery.

Table 3: Drug Loading, Encapsulation Efficiency, and Drug Content

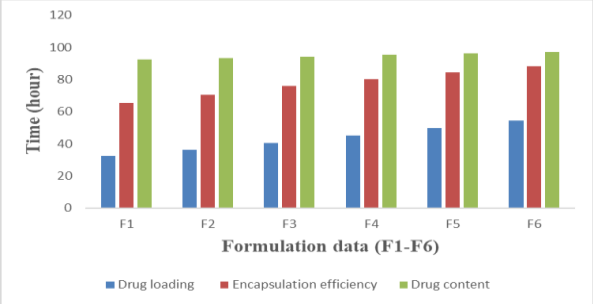

Fig 5. Data shown for Drug loading, Encapsulation efficiency(%) and Drug Content (%)

In Vitro Dissolution Study of Besifloxacin Hydrochloride-Loaded Nanoparticles

The in vitro dissolution study of Besifloxacin Hydrochloride-loaded nanoparticles was carried out to evaluate the drug release behavior from the nanoparticle system over a specified period of time. The study helps in determining the sustained release characteristics of the developed formulation and its suitability for ocular drug delivery. The dissolution study was performed using the dialysis membrane diffusion technique. A pre-soaked dialysis membrane was mounted between the donor and receptor compartments of the diffusion apparatus. The receptor compartment was filled with phosphate buffer saline (PBS, pH 7.4), which served as the dissolution medium and was maintained at 37 ± 0.5°C under continuous magnetic stirring to simulate physiological ocular conditions. An accurately measured quantity of Besifloxacin Hydrochloride-loaded nanoparticle suspension, equivalent to the required dose of drug, was placed in the donor compartment. At predetermined time intervals, aliquots of the dissolution medium were withdrawn and replaced

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with an equal volume of fresh medium to maintain sink conditions throughout the experiment. The withdrawn samples were analysed using a UV-Visible spectrophotometer at 290 nm, and the cumulative percentage drug release was calculated. The results showed that the nanoparticle formulations exhibited a sustained release pattern, indicating controlled release of Besifloxacin Hydrochloride from the polymeric matrix. The sustained release may be attributed to the diffusion of drug through the Eudragit RL100 polymer matrix and the gradual erosion or relaxation of the nanoparticle structure.

Table 4: In vitro drug release data of besifloxacin loaded nanoparticle

Time (hrs)	F1	F2	F3	F4	F5	F6
0.5	25.12	22.84	20.42	19.36	18.78	18.24
1	35.48	32.15	29.84	28.42	27.16	26.38
2	48.26	44.12	41.26	39.84	38.96	38.56
4	62.45	58.36	55.24	53.18	52.04	51.72
6	71.84	68.25	64.32	62.14	61.26	60.84
8	78.62	74.36	70.45	68.12	67.84	67.45
12	85.74	80.62	75.82	73.14	72.65	72.18
24	92.18	88.45	84.26	82.14	81.84	81.36

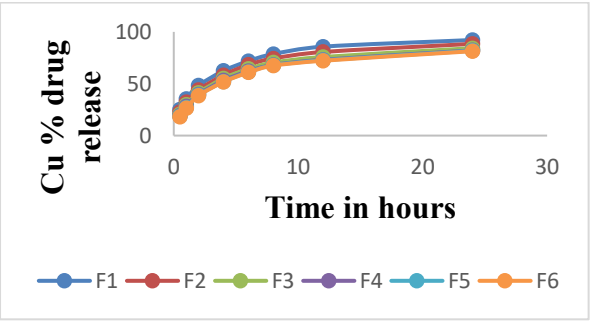

Fig 6. Data shown for the in vitro dissolution study of besifloxacin-loaded nanoparticles

Characterisation of Besifloxacin Hydrochloride Nano In Situ Gel Formulations

The pH of all formulations ranged from 4.01 ± 0.03 to 4.07 ± 0.01, indicating minimal variation among batches. Although slightly acidic, the pH remained within an acceptable range for ophthalmic preparations when buffered by tear fluid upon administration. Maintaining a consistent pH across formulations suggests uniform composition and ensures formulation stability without significant risk of ocular irritation. The sol–gel transition temperature of the formulations was found to decrease progressively from 35 ± 0.4°C (F1) to 29 ± 0.3°C (F6). This reduction can be attributed to the increasing concentration of the thermosensitive polymer, which enhances micelle formation and packing density. Formulations F4–F6 exhibited gelation temperatures closer to ocular surface temperature (~32–34°C), indicating their suitability for rapid in situ gel formation upon instillation. Lower gelation temperatures are advantageous as they ensure immediate phase transition, improving drug retention at the site of action.

Viscosity measurements revealed a significant temperature-dependent increase in all formulations. At 25°C, viscosity ranged from 305 ± 15 cps to 368 ± 40 cps, ensuring ease of instillation in the sol state. Upon increasing the temperature to 35°C, viscosity increased markedly, ranging from 7642 ± 124 cps to 14942 ± 214 cps, confirming the formation of a stable gel matrix.

The progressive increase in viscosity from F1 to F6 indicates enhanced gel strength with higher polymer concentration. This property is critical in prolonging precorneal residence time and minimizing drug loss through nasolacrimal drainage. Among the formulations, F5 and F6 demonstrated the highest viscosity, suggesting superior retention capability. All formulations were found to be clear and transparent, with no visible particulate matter. Clarity is a critical parameter for ophthalmic formulations, as any turbidity may impair vision and reduce patient compliance. The observed transparency confirms uniform dispersion of formulation components and suitability for ocular application (Table 5).

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Table 5: Evaluation parameters of Besifloxacin Hydrochloride Nano In Situ Gel Formulations

Parameter	F1	F2	F3	F4	F5	F6
pH (±SD)	4.01 ± 0.03	4.02 ± 0.02	4.03 ± 0.02	4.04 ± 0.02	4.05 ± 0.03	4.07 ± 0.01
Gelation Temp (°C ±SD)	35 ± 0.4	34 ± 0.3	33 ± 0.5	32 ± 0.2	31 ± 0.4	29 ± 0.3
Viscosity (25°C, cps)	305 ± 15	312 ± 25	322 ± 30	335 ± 29	350 ± 50	368 ± 40
Viscosity (35°C, cps)	7642 ± 124	8500 ± 150	9600 ± 180	10252 ± 128	12000 ± 300	14942 ± 214
Clarity	Clear	Clear	Clear	Clear	Clear	Clear

In-vitro drug release study of besifloxacin nano in situ gel:

The in vitro drug release study of formulations F1–F6 demonstrated a time-dependent increase in cumulative drug release, as presented in Table 6. All formulations exhibited a progressive release pattern, indicating effective diffusion of the drug from the nano in situ gel system.At the initial time points (0.5–1 h), a relatively faster release was observed across all formulations, which may be attributed to the release of drug present on or near the surface of the nanoparticles and the partially hydrated gel matrix. This initial release phase is beneficial for achieving a rapid onset of therapeutic action. As time progressed, the release rate became more controlled, suggesting a transition from initial burst release to a diffusion-controlled mechanism. The presence of Eudragit RL 100 nanoparticles and the thermosensitive gel matrix likely contributed to this sustained release behavior by forming a barrier that regulates drug diffusion. A comparative analysis among formulations revealed that drug release increased progressively from F1 to F6. At 6 hours, formulation F6 exhibited the highest cumulative drug release (91.7%), followed by F5 (90.4%) and F4 (89.1%). The enhanced release observed in F6 may be attributed to the optimized polymer composition and gel structure, which facilitated efficient drug diffusion while maintaining matrix integrity. The improved performance of F5 and F6 formulations suggests that an optimal balance between polymer concentration and gel strength plays a critical role in modulating drug release. Higher polymer concentrations likely enhanced gel network formation, improving drug retention initially, followed by sustained and near-complete release over time. In contrast, formulations F1–F3 showed comparatively lower drug release, which may be due to less efficient gel formation and weaker matrix structure, leading to suboptimal diffusion characteristics. The release profile indicates that the developed nano in situ gel system follows a controlled release mechanism, likely governed by diffusion through the polymeric matrix. Among all formulations, F6 was identified as the optimized formulation, as it provided the highest cumulative drug release within 6 hours along with a desirable sustained release pattern.

Table 6: In-vitro drug release data of besifloxacin nano in situ gel

Time (h)	F1 (%CDR)	F2 (%CDR)	F3 (%CDR)	F4 (%CDR)	F5 (%CDR)	F6 (%CDR)
0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	22.4	24.1	26.3	28.8	31.2	35.6
1	34.8	37.5	40.9	44.6	49.2	54.8
2	48.2	52.6	57.1	62.4	68.8	74.9
3	58.6	63.1	68.2	73.6	79.4	84.8
4	67.3	71.5	76.2	81.4	86.0	88.9

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5	73.9	78.2	82.5	86.7	89.4	90.6
6	79.8	83.4	86.8	89.1	90.4	**91.7**

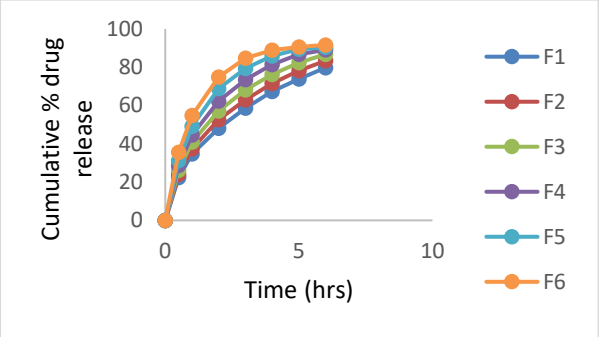

Fig 7: Data shown for the in vitro dissolution study of besifloxacin-loaded in-situ gel

CONCLUSION:

The present study successfully developed and characterized a besifloxacin hydrochloride-loaded nanoparticle incorporated ion-sensitive in situ ophthalmic gel for the effective treatment of keratoconjunctivitis. Nanoparticles prepared using Eudragit RL100 demonstrated desirable physicochemical properties, including nanoscale particle size, narrow size distribution, high zeta potential, and satisfactory encapsulation efficiency, indicating good stability and efficient drug loading. The incorporation of nanoparticles into a gellan gum-based ion-sensitive in situ gel system resulted in formulations with appropriate clarity, acceptable pH, and thermoresponsive behavior suitable for ocular administration. The sol–gel transition occurred at physiological conditions, ensuring ease of instillation followed by rapid gelation at the ocular surface. Viscosity studies confirmed the formation of a stable gel matrix, which is essential for prolonging precorneal residence time and reducing drug loss. In vitro drug release studies demonstrated a sustained and controlled release profile for all formulations, with formulation F6 exhibiting the highest cumulative drug release and overall optimal performance. The improved release behavior and physicochemical characteristics of F6 can be attributed to the optimized polymer concentration and enhanced gel structure. The developed nanoparticle-based ion-sensitive in situ gel system offers a promising approach for ocular drug delivery by enhancing drug bioavailability, prolonging residence time, and reducing dosing frequency. This formulation strategy has the potential to improve therapeutic outcomes and patient compliance in the treatment of ocular infections.

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