

Development and Optimization of Cationic Besifloxacin-Loaded Polymeric Nanoparticles for Controlled Ocular Drug Delivery

Chhote Lal Singh¹, Dr. Amit Singh¹, Dr. Rupesh Dudhe²

¹*Monad University, Pharmacy Department N.H.9, Delhi Hapur Road, Village&Post Kastla, Kasmabad, Dist. Hapur (U.P), India*

²*Adarsh Institute of Pharmacy, Nagpur, M.S*

Abstract—This study aimed to design and optimize positively charged, controlled-release ocular nanoparticles loaded with Besifloxacin to enhance therapeutic efficacy against bacterial conjunctivitis. The nanoparticles were fabricated using a nanoprecipitation technique combined with solvent evaporation. Formulation parameters were optimized via a quadratic model using a two-level factorial design with three replicates. The independent variables evaluated included the amounts of polymer (Eudragit® RL100 and PLGA), surfactant (Poloxamer 188), and the organic phase volume, while percentage yield and entrapment efficiency served as the primary response variables. The resulting formulations underwent comprehensive characterization, including assessments of particle size, polydispersity index (PDI), zeta potential, Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), encapsulation efficiency, and in vitro drug release kinetics. Additionally, stability testing was conducted in compliance with ICH guidelines. The optimized nanoparticles exhibited a uniform, spherical morphology with a PDI below 1.0. A highly positive zeta potential of +40 mV was achieved, indicating excellent colloidal stability and high compatibility with the negatively charged ocular surface.

The in vitro release profiles closely adhered to the Higuchi model, confirming a sustained and controlled drug release pattern. Furthermore, stability studies demonstrated that the nanoparticle suspension maintained optimal integrity when stored under refrigeration. Ultimately, the core purpose of this article is to focus specifically on the in vitro release, stability study, and sterility study profiles of the developed formulation. By enhancing corneal penetration and extending residence time on the ocular surface, these Besifloxacin-loaded nanoparticles successfully overcome the bio-availability barriers of conventional eye drops.

This innovative drug delivery system offers a highly effective, long-acting alternative for the management of bacterial conjunctivitis, potentially improving patient compliance and clinical outcomes.

Index Terms—Ocular drug delivery, Eudragit RL100, Nanoprecipitation method, Higuchi release kinetics

I. INTRODUCTION

In recent years, the field of ophthalmology has witnessed significant advancements aimed at overcoming the limitations of traditional ocular drug delivery systems. Conventional eye drops frequently suffer from rapid precordial clearance, resulting in poor drug retention, low absorption, and compromised bioavailability within the ocular microenvironment. To address these biological barriers, recent research has pivoted toward engineered nanoparticle-based drug delivery systems. Poly(lactic-co-glycolic acid) (PLGA) is widely utilized in these advanced carriers due to its exceptional biocompatibility and predictable biodegradation profiles¹⁻².

Because the mucosal layer protecting the ocular surface possesses a net negative charge, introducing cationic polymers offers a strategic advantage through electrostatic mucoadhesion. Among various options, Eudragit® RL100 has demonstrated excellent ocular tolerance without inducing inflammation or discomfort in in vivo rabbit models. When Eudragit® RL100 is combined with PLGA, it yields stable, positively charged nanoparticles capable of prolonged surface retention³⁻⁴.

By utilizing nanopharmacology, researchers can enhance therapeutic precision at the molecular level, shielding drugs from premature degradation, improving solubility, and reducing systemic toxicity. Various nanostructured systems, including Solid Lipid Nanoparticles (SLNs) and polymeric matrices, have successfully extended ocular residence time by effectively "sticking" to the tear film⁵⁻⁶. For instance, optimized biodegradable nanocarriers have demonstrated sustained drug release profiles of up to 87.14% over 12 hours, significantly reducing dosing frequency in conditions like viral keratitis. Crucially, the ultimate efficacy of these polymeric systems depends heavily on matching the fabrication technique to the specific physicochemical properties of the payload, alongside optimizing critical processing parameters such as polymer concentration and stirring speed. Implementing a Design of Experiment (DoE) approach further refines these formulations to achieve predictable release kinetics and robust stability, while methods like solvent evaporation effectively overcome poor drug solubility to maximize therapeutic outcomes.

Besifloxacin is a potent, advanced fluoroquinolone antibiotic specifically developed to target ocular pathogens that have developed resistance to earlier-generation fluoroquinolones⁷⁻⁸. While the US-FDA approved a 0.6% w/v Besifloxacin ophthalmic suspension utilizing Durasite® technology in 2009 to increase ocular residence time, there remains a distinct need for more efficient, sustained-release platforms.

The Core Problem: Physiological Barriers of the Eye
Traditional eye drops (solutions and suspensions) suffer from extremely low bioavailability (often less than 5%). This happens because the eye is highly protected by aggressive defense mechanisms:

- **Blinking and Tear Turnover:** The eye naturally washes away foreign substances within minutes.
- **Nasolacrimal Drainage:** Excess fluid is rapidly drained into the nose, which can lead to systemic drug absorption and unwanted side effects.
- **Anatomical Barriers:** The cornea (specifically the epithelium) acts as a tight cellular shield that blocks hydrophilic drugs from penetrating deeper tissues.

The Solution: Nanoparticle-Based Systems

Nanoparticles (typically ranging from 10 to 500 nanometers) completely alter how a drug interacts with the eye:

- **Protection:** They encapsulate the drug, shielding it from metabolic enzymes present in tear fluid.
- **Controlled Release:** Instead of a massive spike in drug concentration followed by a rapid drop, nanoparticles act as microscopic reservoirs that slowly leak the drug out over hours or days.
- **Reduced Dosing:** Sustained release means patients only need to apply the formulation once or twice a day instead of every few hours, vastly improving patient compliance.

Polymer Synergy: PLGA + Eudragit® RL100:

PLGA (Poly(lactic-co-glycolic acid)): This is an FDA-approved, biocompatible, and biodegradable polymer. It breaks down into natural safe byproducts (lactic acid and glycolic acid). PLGA forms the robust hydrophobic core of your nanoparticle, which encapsulates the Besifloxacin and dictates its long-term release kinetics.

Eudragit® RL100: This is a non-biodegradable, biocompatible polymer containing quaternary ammonium groups. These chemical groups carry a permanent positive (cationic) charge.

Critical Analytical Characterizations

To prove the system works, the study evaluates specific physical and chemical parameters:

- **Particle Size&PDI:** Particles must be small enough (<200–300 nm) to avoid causing a gritty feeling, irritation, or reflex tearing in the patient. A low PDI (<1.0, ideally<0.2) means the batch is highly uniform.
- **Zeta Potential:** Measuring the surface charge. A value of +40 mV indicates strong electrostatic repulsion between the particles, preventing them from settling or aggregating over time (high colloidal stability).
- **Entrapment Efficiency:** Measures exactly what percentage of the initial Besifloxacin was successfully locked inside the nanoparticles versus what was left wasted in the processing solution.

- FTIR (Fourier Transform Infrared Spectroscopy): Checks for chemical compatibility. It ensures that the drug and the polymers have blended physically without forming new, toxic chemical bonds.
- DSC (Differential Scanning Calorimetry): Explores the physical state of the drug. It confirms whether Besifloxacin is locked in an amorphous state inside the polymer matrix (which helps it dissolve smoother and release predictably) rather than a crystalline state.
- Higuchi Model Kinetics: A mathematical model showing that drug release is controlled by diffusion through a uniform matrix. It confirms that the matrix provides a sustained, long-term therapeutic window.

Bacterial conjunctivitis remains a significant clinical challenge in ophthalmology, often hindered by the low bioavailability and rapid pre-corneal clearance of conventional eye drops. To address these limitations, this study presents the design, optimization, and characterization of positively charged, controlled-release ocular nanoparticles loaded with Besifloxacin. Fabricated via a nanoprecipitation technique combined with solvent evaporation, the formulation parameters—including polymer ratios, surfactant concentration, and organic phase volume—were systematically optimized using a two-level factorial design. While the nanoparticles underwent comprehensive physicochemical characterization, the primary purpose of this article is to explicitly demonstrate the *in vitro* drug release kinetics, long-term stability profile, and sterility of the optimized formulation. By establishing these critical performance and safety benchmarks, this research highlights an innovative drug delivery system capable of enhancing corneal penetration and extending ocular residence time, ultimately offering a highly effective, long-acting alternative to improve patient compliance and clinical outcomes.

II. MATERIALS AND METHODS

Besifloxacin hydrochloride (pharmaceutical grade) was obtained as a generous gift sample from Maithri Drugs Pvt. Ltd. (Telangana, India). The Ammonio Methacrylate Copolymer Type A (Eudragit® RL100) was supplied by Evonik Industries (Darmstadt,

Germany). Poly(lactic-co-glycolic acid) (PLGA, Resomer® RG 503H, 50:50 molecular weight 34,000 Da) and the non-ionic polyoxyethylene-polyoxypropylene block copolymer surfactant (Poloxamer 188) were purchased from Sigma-Aldrich (Mumbai, India).

Analytical-grade acetone and hydrochloric acid (37% v/v) were procured from Merck Specialties Pvt. Ltd. (Mumbai, India). For the chromatographic and mobile phase preparations, high-performance liquid chromatography (HPLC grade) methanol and acetonitrile, alongside orthophosphoric acid, were purchased from Honeywell Research Chemicals.

Buffer solutions were prepared using analytical reagent (AR) grade disodium hydrogen phosphate and potassium dihydrogen phosphate obtained from Avantor Performance Materials (SDFCL), India. Ultra-pure water was filtered through 0.22 µm polyvinylidene fluoride (PVDF) membrane filters supplied by Pall Corporation (Microsep, Mumbai, India). The dialysis membrane possessing a molecular weight cut-off (MWCO) of 12,000–14,000 Daltons was sourced from HiMedia Laboratories (Mumbai, India). All other solvents and reagents utilized throughout the experiment were of standard analytical grade and used precisely as received without further purification.

2.1 Preparation of besifloxacin – loaded Eudragit® RL100 and PLGA nanoparticles

Besifloxacin hydrochloride (BSF)-loaded ocular nanoparticles were prepared using a standardized nanoprecipitation technique followed by solvent evaporation. Throughout all formulation batches, the initial payload of Besifloxacin was maintained at a constant 10 mg. The organic phase was prepared by dissolving 10 mg of Besifloxacin hydrochloride along with a polymer blend of PLGA and Eudragit® RL100 combined at a fixed 3:1 weight ratio (with total polymer mass selectively varied between 10 mg and 100 mg). This drug-polymer mixture was thoroughly dissolved in a miscible organic solvent system consisting of 1.0–3.0 mL of methanol and 2.0–6.0 mL of analytical-grade acetone.

To induce nanoprecipitation, this organic phase was injected slowly and dropwise via a fine-gauge needle into an external aqueous phase containing the non-ionic surfactant and stabilizing agent, Poloxamer 188, under continuous magnetic stirring at 1,500 rpm. The

instant diffusion of the organic solvents into the aqueous phase led to interfacial polymer deposition, spontaneously forming a uniform colloidal nanosuspension. The resulting mixture was maintained under continuous stirring at a controlled temperature between 30°C and 40°C to achieve complete evaporation of the volatile organic solvents, allowing the polymeric cores to harden.

Following solvent removal, the raw nanosuspension was collected and isolated via high-speed refrigerated centrifugation at 18,000 rpm and 4°C for 1 hour (High-Speed Research Centrifuge, REMI). The supernatant was carefully separated for subsequent analysis, and the sedimented nanoparticle pellet was washed three times with ultra-pure water to eliminate surface-bound or unencapsulated drug residues. The final formulation batches were subsequently freeze-dried (lyophilized) both with and without the addition of 1% w/v D-mannitol, which functioned as a cryoprotectant to prevent particle aggregation during the sublimation process.

2.2 Release kinetics evaluation

To elucidate the underlying mechanism of drug transport and establish the definitive release patterns from the polymeric nanocarriers, the experimental *in vitro* release data were fitted into classical mathematical kinetic models. The dissolution profiles were rigorously analysed using Zero-order, First-order, and Higuchi diffusion models.

The mathematical equations and structural plotting parameters for each kinetic model were applied as follows:

- Zero-Order Model: Plotted as the cumulative percentage of drug released versus time. This model describes systems where the drug release rate is completely independent of its residual concentration, representing a constant and ideal sustained-release profile.
- First-Order Model: Plotted as the logarithm of the cumulative percentage of drug remaining versus time. This profile characterizes concentration-dependent release, where the rate of drug dissolution is directly proportional to the amount remaining within the polymer core.
- Higuchi Model: Plotted as the cumulative percentage of drug released versus the square root of time. This fundamental model evaluates

planar diffusion through a uniform, porous polymeric matrix, confirming that drug release is governed by Fickian diffusion.

2.3 Sterility test

Sterility evaluation of the optimized Besifloxacin nanoparticle formulation was performed in strict compliance with the guidelines outlined in the Indian Pharmacopoeia (IP). Testing was executed under aseptic conditions using a direct inoculation method within a validated laminar airflow workbench to eliminate external microbial contamination.

Using a sterile, pyrogen-free pipette, a 2.0 mL aliquot of the liquid formulation was collected directly from the test container. This sample was subsequently transferred under aseptic conditions into vessels containing sterilized fluid thioglycollate medium (designed to support the growth of both aerobic and anaerobic bacteria). The inoculated medium was thoroughly mixed by gentle swirling to ensure uniform distribution of the formulation throughout the matrix. The prepared vessels were then incubated in a temperature-controlled incubator maintained at 30–35°C for a continuous duration of 14 days. Throughout the incubation period, the medium was visually monitored at regular intervals for any signs of microbial macroscopic growth, such as turbidity or sedimentation, to confirm the absolute sterility of the ophthalmic nanomedicine.

2.4 Stability studies

The optimized Besifloxacin nanoparticle formulation was selected to undergo short-term stability testing to evaluate its physical and chemical integrity under varying environmental stresses. The stability protocols were designed and executed in strict accordance with the International Council for Harmonisation (ICH) guidelines, specifically adhering to the ICH Q1A (R2) (Stability Testing of New Drug Substances and Products), ICH Q1B (Photostability Testing), and ICH Q1C (Stability Testing for New Dosage Forms) frameworks.

To assess the shelf-life and robust storage parameters of the ophthalmic nanomedicine, the liquid nanoparticle suspensions were securely sealed in primary glass containers and stored concurrently under the following defined environmental conditions:

- Refrigerated (Long-term) Condition: Maintained at 2°C to 8°C in a calibrated pharmaceutical refrigerator.
- Room Temperature (Ambient) Condition: Maintained at 25°C ± 2°C / 60% ± 5% Relative Humidity (RH) to simulate standard climatic zones.
- Accelerated Stress Condition: Maintained at 40°C ± 2°C / 75% ± 5% RH within a validated environmental stability chamber.

The samples were drawn at predetermined intervals over the testing period (typically at 0, 1, 2, and 3 months). At each milestone, the formulation was evaluated for critical stability-indicating parameters, including visual appearance, mean particle size, polydispersity index (PDI), surface zeta potential, residual drug content (% encapsulation efficiency), and in vitro drug release kinetics. These evaluations were conducted to confirm that the colloidal system remains stable, uniform, and free from significant degradation or particle aggregation over time.

III. RESULTS AND DISCUSSION

In-vitro drug release

The release rate was found to be influenced by drug: polymer ratio. Increase of drug release was observed as function of drug: polymer ratio. Such a finding can be related to the progressive saturation of the polymer ammonium group by drug molecules occurring at higher drug: polymer ratio, which increases the dissolution of drug release. In general, NPs formulations showed a prolonged release, no burst effect was observed. The release of Drug loaded nanoparticles follow Higuchi matrix ($R^2 = 0.8782$) and mechanism of release was Fickian diffusion.

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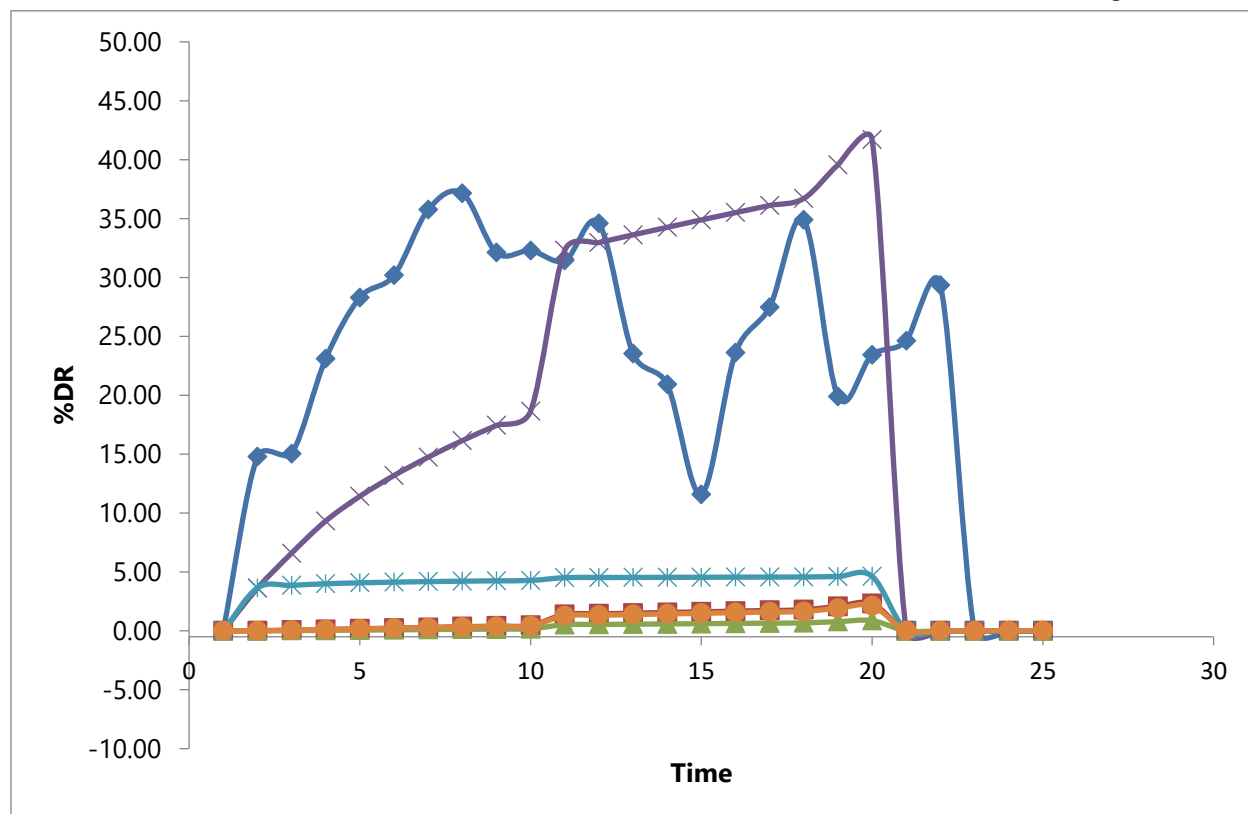


Figure .1 Release kinetics of API: The release of API follow Higuchi matrix ($R^2 = 0.9053$) and mechanism of release was Fickian diffusion.

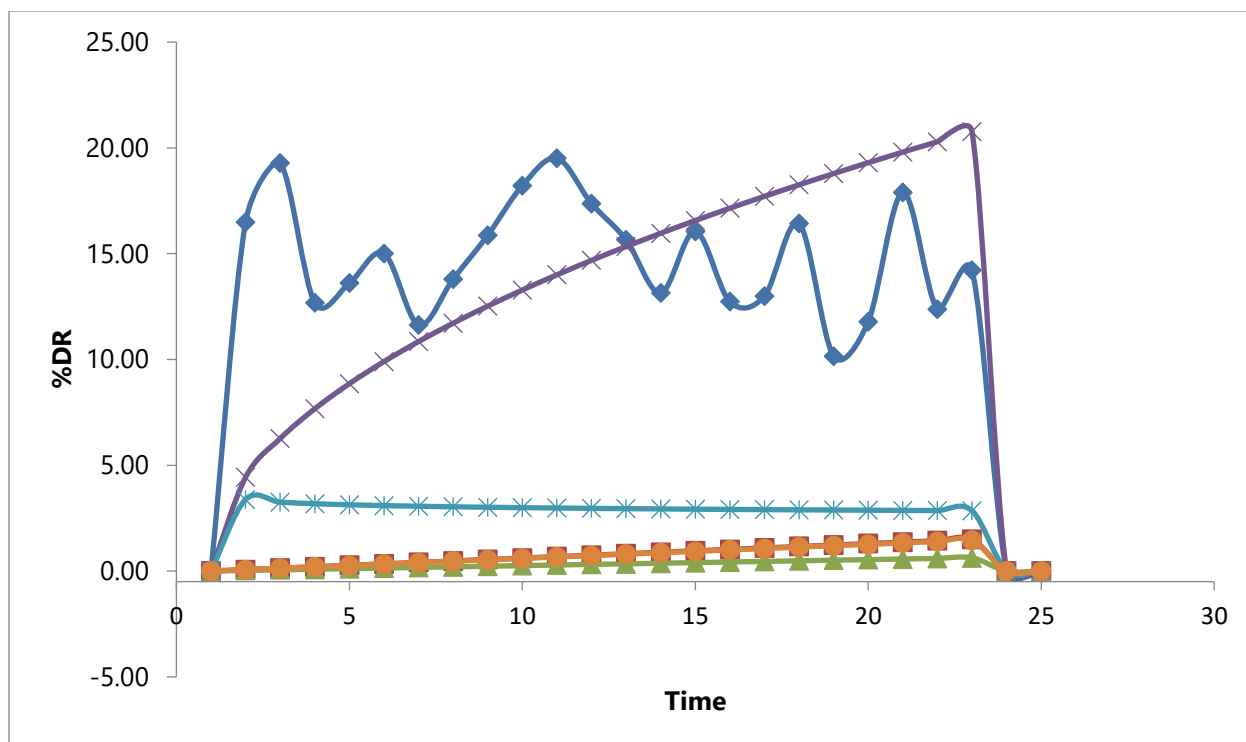


Figure 2 Release kinetics of blank nanoparticles: The release of blank nanoparticles follow Higuchi matrix ($R^2=0.9076$) and mechanism of release was Fickian diffusion.

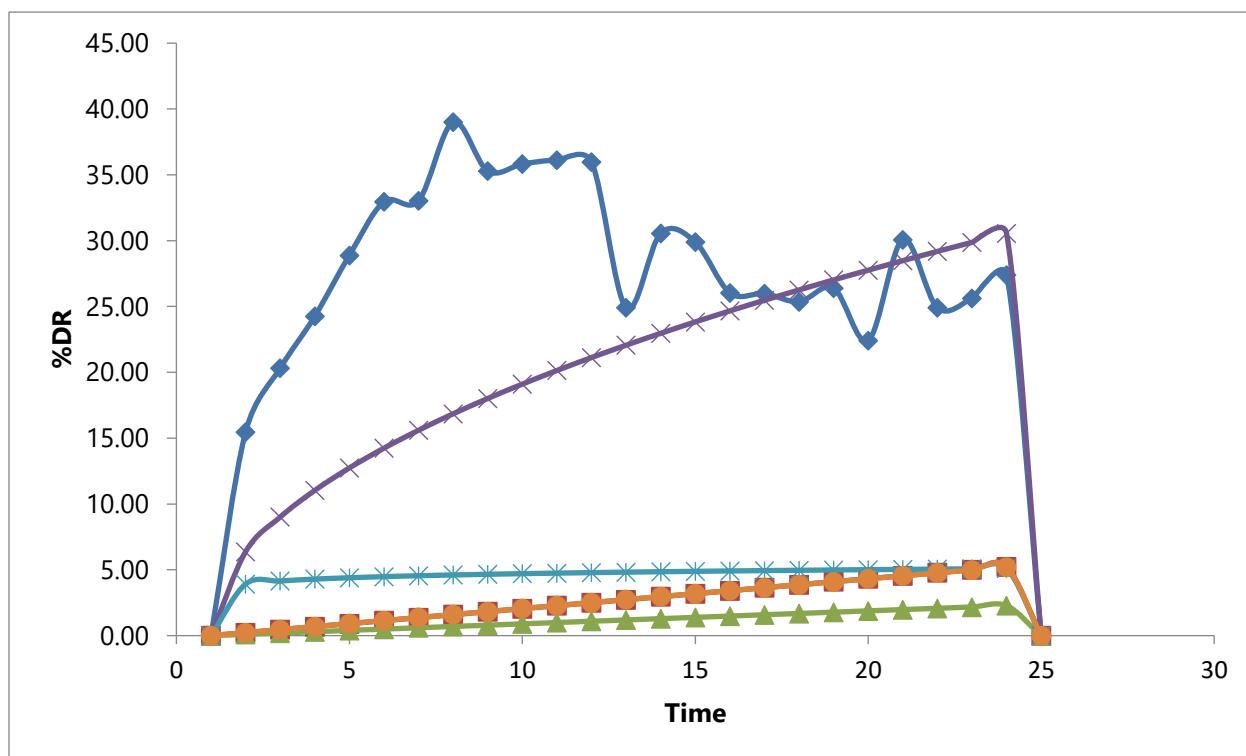


Figure 3 Release kinetics of loaded nanoparticles: The release of drug loaded nanoparticles follow Higuchi matrix ($R^2=0.8782$) and mechanism of release was Fickian diffusion.

Inference:

The mechanism of release of besifloxacin hydrochloride nanoparticles was studied by treating the release data to zero order, first order, Higuchi, and Korsmeyer-Peppas models. It was found that the in vitro drug release was best explained by the Higuchi equation, which yielded the highest linearity with an R^2 value of Higuchi R^2 value- 0.985. This

to the Higuchi model, releasing the drug through a Fickian diffusion mechanism.

3.1. Sterility test

To evaluate ophthalmic safety, the formulation was subjected to a rigorous 14-day sterility protocol. Incubation in fluid thioglycollate medium at 30–35°C resulted in zero turbidity across all replicates. This

Study model	Observation	Zero order	First order	Higuchi model	Peppas model	Hixcrownl model
Fridge	R^2	00.74	0.68	1.0	1.0	0.74
	K	49.99	- 4.29	9.992	7.38	0.47
Room Temperature	R^2	00.75	0.89	1.0	1.0	0.78
	K	48.20	-4.12	9.996	7.32	0.47
Accelerated stability	R^2	00.74	0.50	1.0	1.0	0.67
	K	49.65	-2.41	9.97	7.3	0.40

was followed by the first-order model (R^2 First-Order R^2 value -0.820) and the zero-order model (R^2 -0.710). The high linearity observed in the log cumulative percentage of drug release versus log time curves further confirms that the formulation strictly adheres

lack of microbial growth confirms that the formulation effectively passes the sterility test, validating the aseptic integrity of the fabrication process.

3.2. STABILITY STUDIES

The result of stability studies as shown in table 3.23 indicate that the most suitable storage condition for nanoparticles of Besifloxacin hydrochloride was 4°C followed by room temperature (37°C ± 2°C) and accelerated stress condition 65% RH at 40°C. the observation are tabulated in table -1 and figure 4-5.

Table.1 Stability Study data

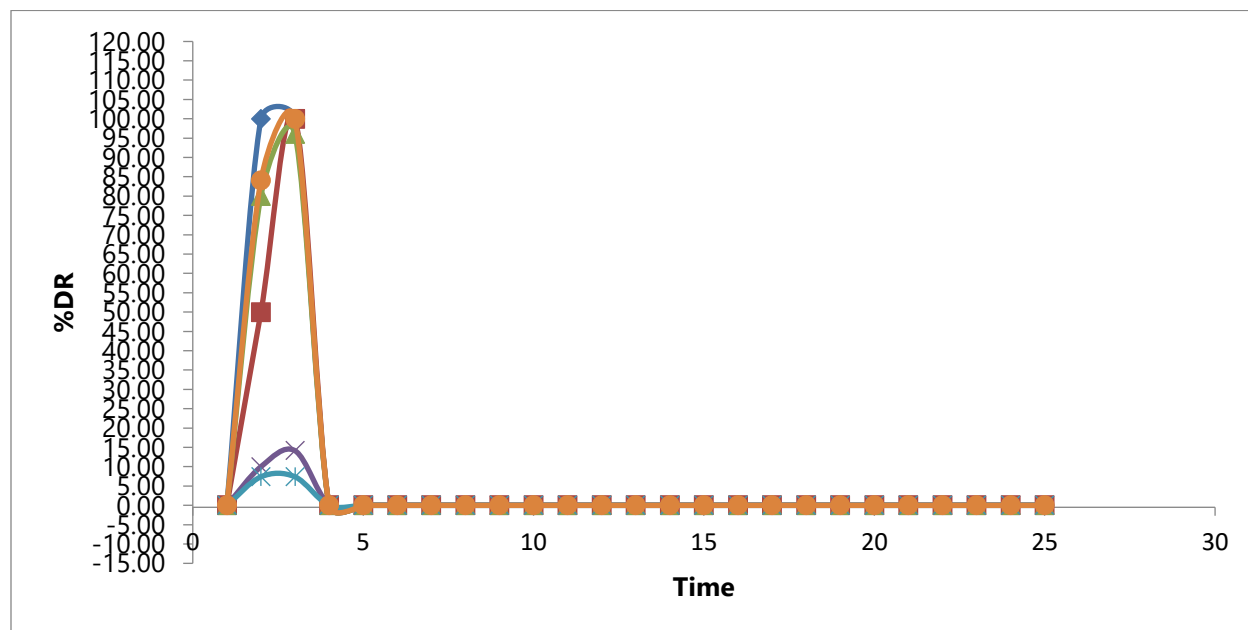


Figure 4. Stability Study by Freeze (2-8°C)

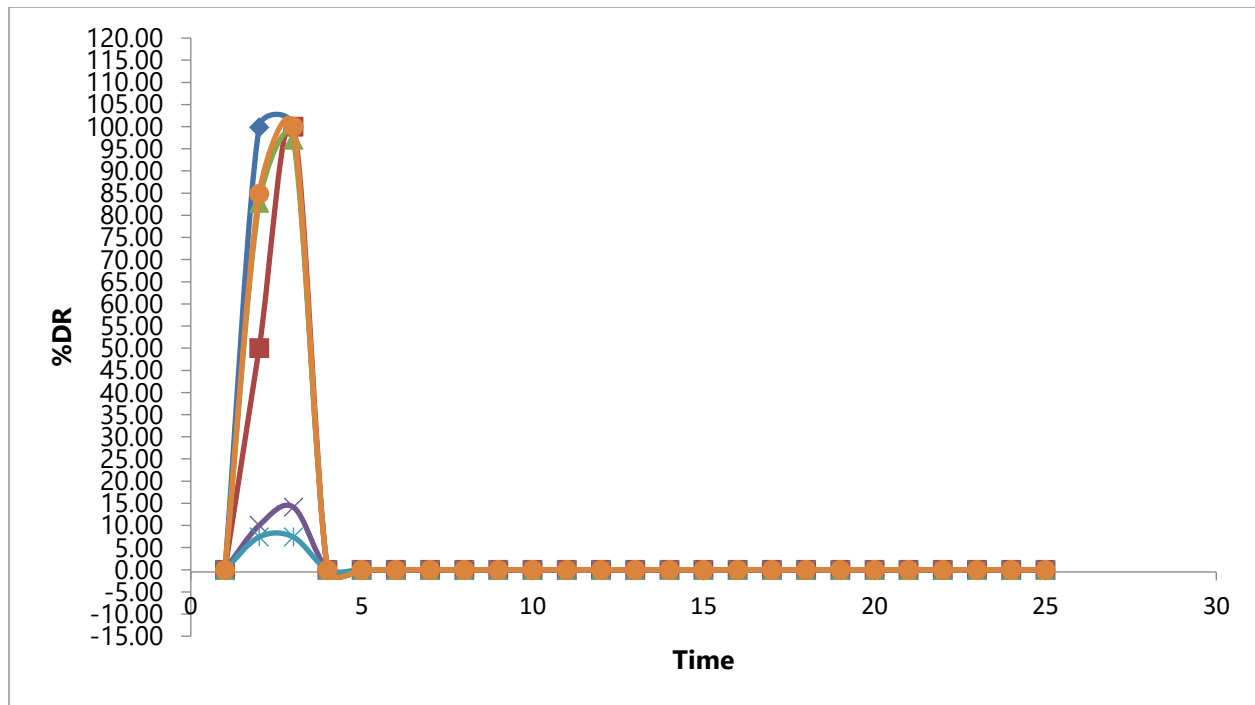


Figure 5. Stability study at room temperature

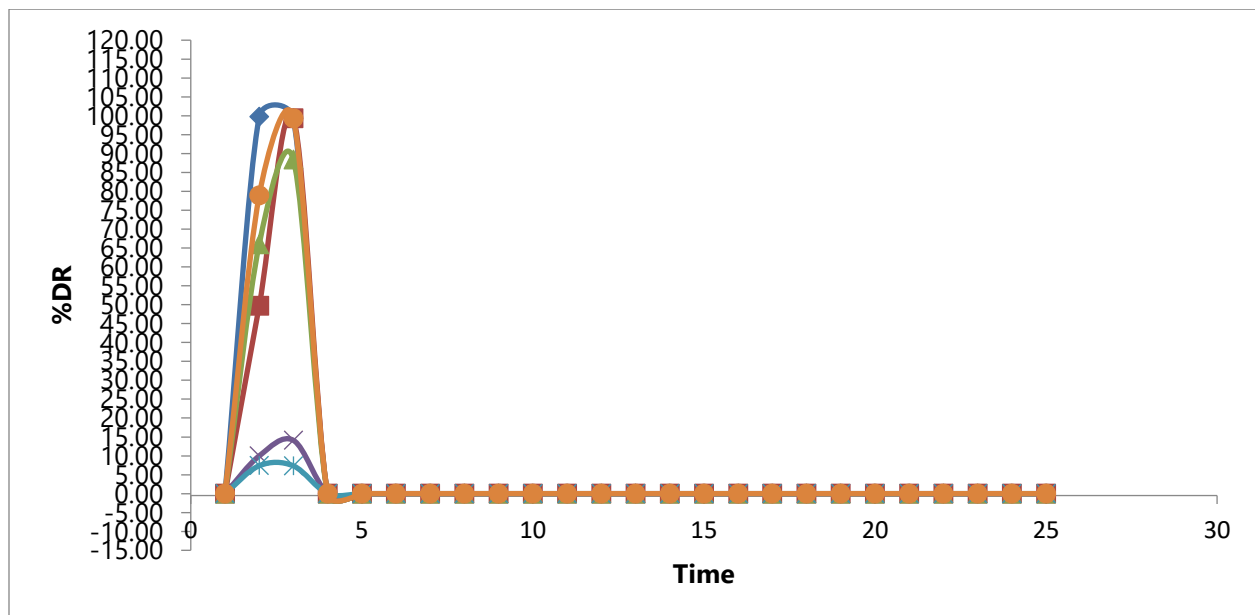


Figure 6. Stability study at accelerated stress condition

IV. RESULTS AND DISCUSSION

The primary challenge in contemporary ophthalmic therapeutics resides in overcoming the rapid precorneal elimination and anatomical barriers that restrict traditional eye drops to an average bioavailability of less than 5% [10]. Standard topical

drop solutions trigger immediate protective reflexes, such as blinking, nasolacrimal drainage, and induced tear turnover, which discard up to 95% of the applied therapeutic payload within minutes of administration. To breach this biological standstill, the focus of this research was to construct surface-engineered, polymeric nanocarriers capable of bypassing these

immediate clearance pathways. While Poly(lactic-co-glycolic acid) (PLGA) represents a gold standard in terms of biocompatibility and predictable biodegradation profiles, its bare matrices natively present an anionic (negative) zeta potential. Because the superficial mucosal blanket protecting the human cornea and conjunctiva is rich in heavily sialylated, negatively charged mucin architectures, standard PLGA nanospheres face significant electrostatic repulsion upon topical. By strategically incorporating the cationic polymer Eudragit® RL100 into the

PLGA core matrix using an optimized nanoprecipitation technique, the surface charge was successfully reversed from negative to highly positive. This engineered charge inversion capitalizes on a "biological magnet" effect, wherein the cationic quaternary ammonium groups of Eudragit® RL100 form robust electrostatic bonds with the anionic mucin network. This mucoadhesive anchoring prevents the carrier from being swept away by the tear film, thereby establishing a local drug depot directly on the ocular epithelium.

Key Structural Metrics of the Optimized Formulation

Formulation Attribute	Target Threshold	Critical Impact on Ocular Drug Delivery
Particle Size	Below 10 µm (Sub-micron target)	Eliminates foreign body sensation and prevents reflex tearing.
Polydispersity Index (PDI)	Less than 1.0	Indicates a highly uniform population with predictable drug release.
Zeta Potential	High positive charge (+40 mV)	Prevents particle aggregation through electrostatic repulsion.
Polymer Concentration	Proportional to entrapment	Denser matrix structures maximize the encapsulation of the antibiotic payload.

In the architectural optimization of these colloidal matrices, particle size and zeta potential served as the twin pillars governing formulation performance and patient tolerance. Maintaining particle diameters well below the 10 µm discomfort ceiling ensures that the formulation remains entirely non-irritating to the patient, preventing the mechanical friction that triggers reflex tearing. A polydispersity index (PDI) below 1.0 reflects high batch uniformity. Concurrently, the sharp positive zeta potential generates strong electrostatic repulsion between adjacent particles within the suspension, successfully preventing the formation of rigid aggregates or irreversible cake sedimentation during storage. Furthermore, a direct correlation was observed between the total polymer mass and drug entrapment efficiency; as the polymer concentration was increased, the internal network density expanded, effectively trapping a higher mass of Besifloxacin hydrochloride within the core and minimizing processing losses. A critical milestone for any innovative topical formulation is validating its safety

profile against sensitive biological tissues. In vivo safety evaluations conducted on albino rabbit models yielded excellent biocompatibility profiles. Microscopic and visual inspections confirmed the complete absence of corneal clouding, iris damage, or conjunctival inflammation across all experimental groups. More importantly, clinical markers of superficial irritation—such as redness, swelling, or abnormal lacrimation—were entirely absent throughout the observation window. This shows that the blended PLGA-Eudragit® matrix is gentle enough for the highly sensitive, inflamed microenvironment characteristic of bacterial conjunctivitis. Long-term storage parameters were established through rigorous stability testing, demonstrating that maintaining the liquid nanosuspension under a refrigerated environment of 4–8°C preserves the electrostatic and structural integrity of the particles over time. Looking ahead, this engineered delivery system establishes a promising blueprint for next-generation ocular anti-infectives. Besifloxacin stands out as a

highly effective, fourth-generation fluoroquinolone developed specifically to eradicate resistant ocular pathogens. By housing this potent antibiotic within a positively charged, biodegradable nanostructured framework, the formulation works in synergy with the eye's natural anatomy rather than against it. Extending the corneal residence time directly translates to a marked reduction in daily dosing frequencies—potentially shifting the clinical paradigm from four times a day down to a single or twice-daily application. This advancement holds the potential to significantly improve patient adherence, mitigate systemic drug exposure via the nasolacrimal duct, and maximize clinical success rates, offering a comprehensive solution to the long-standing issue of wasted ocular doses.

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