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"DEVELOPMENT OF NATURAL POLYMER BASED ANTIMICROBIAL MICROSPHERES DRUG CIPROFLOXACIN"

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ABSTRACT

The present study aimed to develop and evaluate natural polymer-based antimicrobial microspheres containing Ciprofloxacin for sustained drug delivery and enhanced antibacterial activity. Preformulation studies confirmed the purity, stability, and suitability of the drug for microsphere formulation. Microspheres were prepared by the emulsification solvent evaporation method using natural polymers. Among the prepared formulations (MSF1–MSF5), MSF4 exhibited the smallest particle size, highest zeta potential, and maximum entrapment efficiency, indicating superior stability and drug incorporation. SEM analysis showed that the microspheres were nearly spherical with rough surface morphology. The optimized formulation demonstrated sustained drug release over 13 hours with cumulative drug release of 91.37% and followed First-order release kinetics ($R^2 = 0.947$). Antibacterial studies against Escherichia coli revealed that the optimized microsphere formulation exhibited significantly greater antimicrobial activity compared with pure Ciprofloxacin solution. Overall, the developed natural polymer-based Ciprofloxacin microspheres showed promising potential as an effective controlled drug delivery system for improved antimicrobial therapy.

Keywords: Ciprofloxacin, Microspheres, Natural polymers, Antimicrobial activity, Sustained drug release, Emulsification solvent evaporation method, FTIR, SEM, Drug delivery system.

1. INTRODUCTION

Antimicrobial drug delivery systems have gained considerable importance in modern pharmaceutical research because of the increasing prevalence of infectious diseases and the emergence of multidrug-resistant microorganisms **(Loretz *et al.,* 2021).** Conventional dosage forms of antibiotics often require frequent administration due to rapid drug elimination, resulting in fluctuating plasma drug concentrations, reduced patient compliance, and increased risk of microbial resistance. Therefore, the development of controlled and sustained release drug delivery systems has become essential to improve therapeutic efficacy and minimize side effects **(Moser *et al.,* 2019).**

Microspheres are one of the most widely investigated multiparticulate drug delivery systems for controlled release applications. Microspheres are small spherical particles generally ranging from 1 μm to 1000 μm in size, in which the drug is either uniformly dispersed or encapsulated within a polymeric matrix **(Lengyel *et al.,* 2019).** These systems provide several advantages such as prolonged drug release, enhanced bioavailability, reduced dosing frequency, improved stability, and targeted delivery. Microspheres also help maintain therapeutic drug concentration

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for extended periods, thereby improving treatment outcomes in antimicrobial therapy **(Szczeblinska *et al.,* 2017).**

In recent years, natural polymers have attracted significant attention in the formulation of microspheres because of their biocompatibility, biodegradability, non-toxicity, eco-friendliness, and cost-effectiveness **(Idrees *et al.,* 2020).** Natural polymers such as chitosan, sodium alginate, gelatin, guar gum, xanthan gum, and starch possess excellent film-forming and mucoadhesive properties, making them suitable carriers for sustained drug delivery systems. These polymers can encapsulate antimicrobial drugs effectively and control their release over prolonged durations. In addition, natural polymers are generally safer than synthetic polymers and exhibit minimal adverse effects **(Cazorla-Luna *et al.,* 2021).**

Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic widely used for the treatment of various bacterial infections including urinary tract infections, respiratory tract infections, gastrointestinal infections, skin infections, and bone infections. It acts by inhibiting bacterial DNA gyrase and topoisomerase IV enzymes, thereby preventing bacterial DNA replication and leading to bacterial cell death. Ciprofloxacin exhibits potent antibacterial activity against both Gram-positive and Gram-negative microorganisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae **(Gauba and Saxena 2023).**

Despite its therapeutic effectiveness, Ciprofloxacin has certain limitations such as short biological half-life, frequent dosing requirement, gastrointestinal side effects, and variable bioavailability **(Junkert *et al.,* 2024).** Rapid elimination of the drug may result in subtherapeutic concentrations, reducing antimicrobial effectiveness and increasing the possibility of bacterial resistance. Incorporation of Ciprofloxacin into microsphere systems can overcome these limitations by providing sustained and controlled drug release, improving drug stability, reducing dosing frequency, and enhancing antibacterial activity **(Ghumman *et al.,* 2025).**

Characterization of microspheres is an important step in formulation development. Parameters such as particle size, zeta potential, surface morphology, entrapment efficiency, and *in vitro* drug release profile significantly influence the therapeutic performance and stability of the formulation **(Agrawal *et al.,* 2017).** Surface morphology is commonly evaluated using scanning electron microscopy (SEM), while UV spectroscopy and FTIR analysis are used for drug identification and compatibility studies. Drug release kinetics are also evaluated using mathematical models such as Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models to understand the mechanism of drug release **(Askarizadeh *et al.,* 2023).**

Therefore, the present study focuses on the development of natural polymer-based antimicrobial microspheres containing Ciprofloxacin using suitable formulation techniques.

2. MATERIAL AND METHODS

2.1 Chemicals

Ciprofloxacin were obtained from Cipla, a reputable supplier of analytical reagents. Nitta Gelatin India Limited provided the Chitosan and Gelatin. Sodium Alginate was procured from Marine Hydrocolloids (Meron). Polyvinyl Alcohol (PVA), was received from Kuraray India. Tween 80 was acquired from Viswaat Chemicals Ltd. Glutaraldehyde was procured from Acuro Organics Limited. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Lab grade, and Praj Industries Ltd.

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2.2 Pre-formulation studies

Pre-formulation studies are crucial early investigations that assess the mechanical, chemical, and physical characteristics of the active pharmaceutical ingredients (APIs) in Ciprofloxacin. Understanding the drug's stability, solubility, excipient compatibility, and optimal storage conditions is aided by these investigations. Pre-formulation research is essential because it offers vital information required to create a stable, efficient, and secure dosage form **(Ahirwar and Shukla 2023).**

2.2.1 Organoleptic Properties

For Ciprofloxacin, organoleptic evaluation was performed by visually inspecting the color and texture, and by noting any characteristic odor under controlled conditions. **(Eniayewu *et al.,* 2025).**

2.2.2 Solubility study

The solubility study of Ciprofloxacin was performed by adding 1mg of each drug to selected polar and non-polar solvents (such as water, ethanol, methanol chloroform and DMSO) in separate test tubes. Each mixture was vigorously shaken after the settling period; the solutions were visually inspected for clarity and the presence of undissolved drug particles and dissolved particles. A clear solution indicated good solubility, while cloudiness or sediment suggested poor solubility. This method helps determine the solubility profile and guides solvent selection for formulation **(Breda *et al.,* 2009).**

2.2.3 Melting Point

The melting point of the selected drug was determined using approximately 1 mg of the finely powdered drug. This small quantity was carefully packed into a clean capillary tube and placed in the melting point apparatus. The temperature was gradually increased at a controlled rate of 1–2°C per minute, and the temperature range from the onset of melting to complete liquefaction was recorded. The test was repeated three times to ensure accuracy and reproducibility, and the observed melting point was compared with the standard literature value to confirm the drug's identity and purity **(Osonwa *et al.* 2017).**

2.2.4 pH determination

The pH of the drug or formulation was determined using a digital pH meter. This helps to assess the suitability of the drug or formulation for topical or oral application, as pH can influence stability, solubility, and skin compatibility **(Jalil *et al.,* 2015).**

2.2.5 Lambda max determination

The λmax (maximum absorbance wavelength) of the drug was determined using a UV–Visible spectrophotometer to identify the wavelength at which the drug shows maximum light absorption, which is essential for quantitative analysis.

- **Preparation of Standard Stock Solution:**

1. Weigh accurately 1 mg of the drug.
2. Dissolve the drug in a suitable solvent (commonly distilled water, ethanol, or phosphate buffer) to make a 1 mg/mL stock solution.
3. Ensure complete dissolution by gentle stirring or sonication if required.
4. Prepare serial dilutions of the stock **so**lution to obtain concentrations suitable for scanning.

- **λmax Determination Procedure:**

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1. Using the UV–Visible spectrophotometer, scan the prepared solution over a wavelength range of 200–400 nm.
2. Record the absorbance spectrum of the drug.
3. Identify the wavelength at which the highest absorbance occurs. This wavelength is noted as λmax, which will be used for further quantitative estimations, such as calibration curves or drug content analysis.

This method ensures accurate identification of the drug's absorption characteristics, which is critical for stability studies, formulation analysis, and release profiling **(Naveed *et al.,* 2014).**

2.2.5.1 Preparation of Ciprofloxacin standard stock solution in methanol

Accurately weigh 1 mg of Ciprofloxacin and transfers it into a clean volumetric flask. Add 1 mL of methanol to dissolve the drug completely, ensuring a 1 mg/mL stock solution. Mix thoroughly using gentle shaking or vortexing until the Ciprofloxacin is fully dissolved. This stock solution can then be used for further serial dilutions to prepare working solutions for λmax determination, calibration curves, and quantitative analysis of Ciprofloxacin in formulations **(Cazedey *et al.,* 2013).**

2.2.5.2 Linearity and Calibration Curve

A calibration curve of Ciprofloxacin was prepared to determine the linearity of the analytical method. Standard solutions of different concentrations (5–25 µg/mL) were prepared from a 1 mg/mL methanolic stock solution by serial dilution. The absorbance of each solution was measured at the λmax of Ciprofloxacin using a UV–Visible spectrophotometer. A graph of absorbance versus concentration was plotted, which showed a linear relationship obeying Beer–Lambert's law within the selected concentration range. The linear regression equation and correlation coefficient (R^2) were calculated, and the high R^2 value confirmed excellent linearity and reliability of the method. The calibration curve was used as a standard reference for quantitative estimation of Ciprofloxacin in further analytical studies **(Uddin *et al.,* 2022).**

2.2.5.3 Functional group identified by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the functional groups present in Ciprofloxacin and to confirm its chemical integrity. Pure Ciprofloxacin was mixed with potassium bromide (KBr) and compressed into a transparent pellet, which was analyzed over the wave number range of 4000–400 cm^{-1} using an FTIR spectrophotometer. The obtained spectrum showed characteristic absorption peaks corresponding to functional groups such as carboxylic acid (-COOH), carbonyl (C=O), aromatic ring (C=C), and other structural moieties of Ciprofloxacin. The presence of these characteristic peaks confirmed the purity and identity of the drug and provided important information for compatibility and formulation studies **(Qi *et al.,* 2020).**

2.3 Preparation of Ciprofloxacin microsphere Formulation

1. Materials

The drug (Ciprofloxacin) and polymers, namely Hydroxypropyl Methylcellulose (HPMC) and Eudragit RS100, were used for microsphere preparation. Polyvinyl alcohol (PVA) was used as a stabilizer in the aqueous phase. Chloroform served as the organic solvent. All chemicals and reagents used were of analytical grade.

2. Preparation of Ciprofloxacin-Loaded Microspheres

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Ciprofloxacin microspheres were prepared by the emulsification solvent evaporation method. Accurately weighed quantities of HPMC and Eudragit RS100 were dissolved in chloroform to form a clear polymeric solution, into which Ciprofloxacin was dispersed uniformly. An aqueous phase containing 0.2% w/v polyvinyl alcohol (PVA) was prepared separately and maintained under continuous stirring. The drug–polymer solution was then added dropwise into the aqueous phase using a mechanical stirrer at 500 rpm to form an emulsion. Stirring was continued for 3 hours at room temperature to allow complete evaporation of chloroform and formation of rigid microspheres. The prepared microspheres were collected by filtration, washed with deionized water to remove residual PVA and unentrapped drug, dried at room temperature for 24 hours, and stored in a desiccator for further evaluation (**Hariyadi *et al*., 2023)**

Table 1: Composition of microsphere Formulation

Formulation Code	Drug (mg)	HPMC (mg)	Eudragit RS100 (mg)	Chloroform (mL)	PVA (% w/v)	Aqueous Phase (mL)
MSF1	500	50	300	20	0.2%	100
MSF2	500	100	250	20	0.2%	100
MSF3	500	150	200	20	0.2%	100
MSF4	500	200	150	20	0.2%	100
MSF5	500	250	100	20	0.2%	100

2.4 Evaluation parameter of Ciprofloxacin loaded microspheres formulation

2.4.1 Physical properties

The physical properties of the Ciprofloxacin-loaded microsphere formulation can be initially evaluated by visual inspection to assess clarity, color, and homogeneity **(Durgapal *et al.*, 2017).**

2.4.2 Particle size

The particle size of the Ciprofloxacin-loaded microsphere formulation was measured using dynamic light scattering (DLS) with a particle size analyser **(Ghumman *et al.*, 2025).**

2.4.3 Zeta potential

The zeta potential was measured to evaluate the surface charge and stability of the Ciprofloxacin-loaded microsphere formulation, as it reflects the particles' movement velocity in an electric field and helps predict their colloidal stability. In this study, the microsphere samples were diluted tenfold with distilled water and analyzed using the Malvern Zetasizer **(Iranfar *et al.*, 2012).**

2.4.4 SEM analysis

The surface morphology of the optimized Ciprofloxacin-loaded microspheres was examined using scanning electron microscopy (SEM). Before analysis, the microsphere samples were coated with a thin metallic layer of gold or palladium using a sputter coater under vacuum to improve conductivity and prevent charging effects. The coated samples were then exposed to an electron beam, and the emitted secondary electrons generated from the surface interactions were detected to obtain high-resolution images. SEM analysis provided detailed information regarding the shape, surface characteristics, and topography of the microspheres, confirming their morphological properties and structural integrity **(Sahoo, *et al.*, 2021).**

2.4.5 %Entrapment Efficiency

Entrapment efficiency was assessed using an indirect method. The Ciprofloxacin-loaded microsphere formulation was subjected to centrifugation at 1500 rpm for 30 minutes using a

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REMI Ultra Centrifuge. The supernatant, containing the unencapsulated (free) drug, was carefully collected and analyzed using a UV spectrophotometer. The absorbance values obtained were compared against a previously established calibration curve to determine the concentration of the free drug. Entrapment efficiency was then calculated using the following equation: **(Hosny, *et al*., 2010).**

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc. ×100

2.4.6 Drug release study of Optimized microsphere formulation

The *in vitro* drug release study of the formulation was carried out using a Franz diffusion cell apparatus. The formulation was dissolved in phosphate buffer solution (pH 6.8) and placed in the donor compartment, while the receptor compartment contained 50 mL of phosphate buffer maintained at 37 ± 0.5°C under continuous stirring at 50 rpm using a magnetic bead. At predetermined time intervals, 5 mL samples were withdrawn and replaced with fresh phosphate buffer to maintain sink conditions. The withdrawn samples were analyzed spectrophotometrically using a UV–Visible spectrophotometer to determine the amount of Ciprofloxacin released. The obtained release data were further subjected to various kinetic models to evaluate and explain the mechanism and kinetics of drug release from the formulation.

Zero order release F = K0.t F = drug release, K0 = release rate constant, t = release time.

The plot of percentage drug release versus time was linear.

First order release Log (100 – F) = K.t F = drug release, K = release rate constant, t = release time.

A plot of log % drug release versus time was linear.

Higuchi model F = K.t1/2 F = drug release, K = Higuchi constant, t = release time.

A plot of percentage drug release versus square root of time was linear.

Korsmeyer-Peppas model Mt/M∝ = K.tn M = fraction of drug released, K = release constant, t = release time, n = diffusion exponent (**Patel, *et al*., 2022).**

2.5 Anti-Microbial Activity of Microsphere by Well diffusion assay

2.5.1 Nutrient Agar Media Preparation

Nutrient agar medium was prepared to provide a sterile and nutrient-rich environment for the cultivation of non-fastidious microorganisms. Approximately 28 g of nutrient agar powder was dissolved in 1 L of distilled water with gentle heating until completely dissolved, and the pH was adjusted to around 7.0–7.6 if required. The prepared medium was sterilized by autoclaving at 121 °C and 15 psi for 15 minutes to eliminate microbial contamination. After sterilization, the molten agar was aseptically poured into sterile Petri plates under laminar airflow conditions and allowed to cool and solidify. The prepared agar plates were then stored under suitable conditions until further microbiological studies **(Smirnova *et al*., 2023).**

2.5.2 Well Diffusion Assay

The antibacterial activity of Ciprofloxacin-loaded microspheres was evaluated by the agar well diffusion method using Escherichia coli as the test microorganism. Sterile nutrient agar plates were inoculated with bacterial suspension and spread uniformly over the agar surface. Wells of appropriate diameter were prepared aseptically using a sterile cork borer, and different test

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samples including control, free Ciprofloxacin solution, and Ciprofloxacin-loaded microspheres were introduced into the wells. The plates were allowed to stand briefly for diffusion of the samples and then incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition surrounding each well was measured to evaluate the antibacterial activity of the microsphere formulation.

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties of Ciprofloxacin

Table 2: Organoleptic properties of Ciprofloxacin

Drug	Organoleptic properties	Observation
Ciprofloxacin	Color	Pale yellow to light yellow
	Odor	Odorless or faint characteristic
	Appearance	Crystalline powder
	State	Solid

3.1.2 Melting point and pH determination

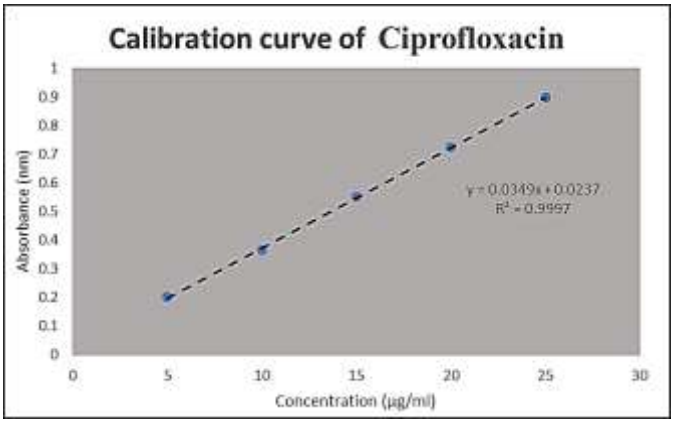
Table 3: Melting point and pH of Ciprofloxacin

Drugs	Observed (Melting point)	Reference (Melting point)	Observed (pH)	Reference (pH)
Ciprofloxacin	256 °C	**255–257 °C**	3.99 pH	3.5 – 4.6 pH

3.2 Calibration curve analysis of Ciprofloxacin drug

Table 4: Calibration curve of Ciprofloxacin

Concentration (µg/ml)	Absorbance (nm)
5	0.203
10	0.365
15	0.550
20	0.724
25	0.897
Mean	**0.5478**
SD	**0.276267**
%RSD	**50.45**



Graph 1: Calibration curve of Ciprofloxacin

3.3 FTIR of Ciprofloxacin

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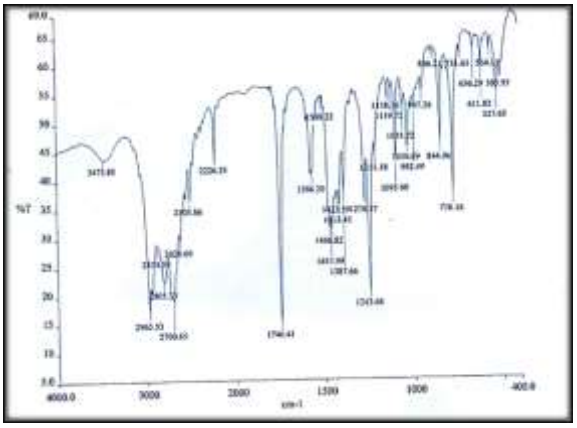


Graph 2: FTIR of Ciprofloxacin

Table 5: Interpretation of IR spectrum of Ciprofloxacin

Peak obtained	Reference peak	Functional group	Name of functional group
3473.85	3500- 3400	N-H stretching	primary amine
2965.53	3000-2840	C-H stretching	alkyne
2874.59	3000-2800	N-H stretching	amine salt
2700.65	2830-2695	C-H stretching	aldehyde
1746.41	1750-1735	C=O stretching	δ-lactone

3.4 Characterization of drug loaded Microsphere formulation

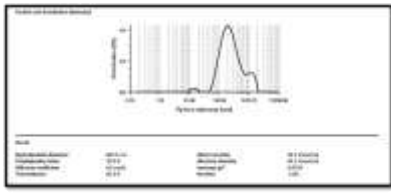
3.4.1 Physical Appearance of Microsphere

Table 6: Physical Appearance of Drug loaded Microsphere

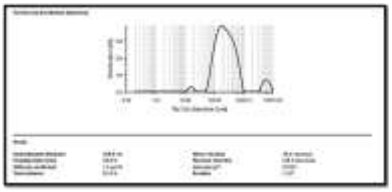
Formulation	Parameters	Observation
Microsphere	Colour	Off-white to pale yellow
	Odour	odorless
	Appearance	Spherical, free-flowing microspheres

Figure 1: Microsphere formulation

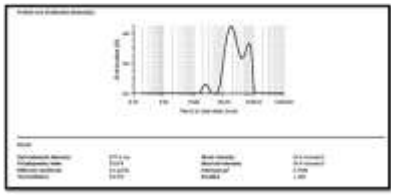
3.4.2 Particle Size



Graph 4: Particle Size MSF1



Graph 5: Particle Size MSF2



Graph 6: Particle Size MSF3

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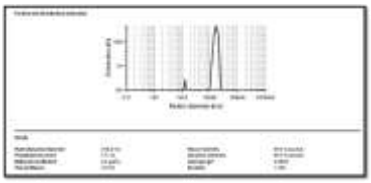
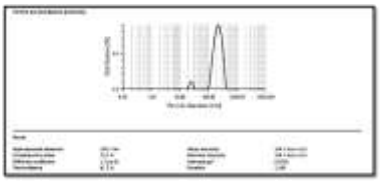
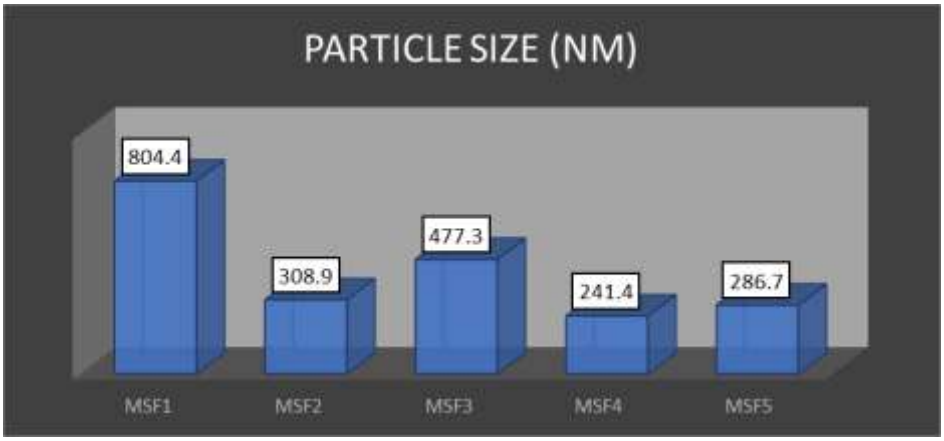


Graph 7: Particle Size MSF4

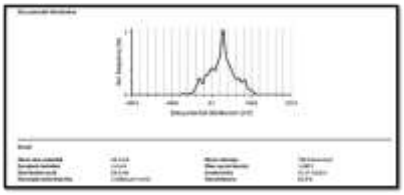


Graph 8: Particle Size MSF5

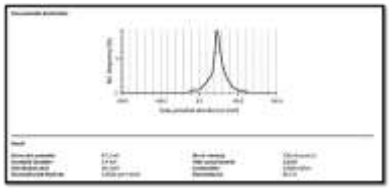


Graph 9: Particle size

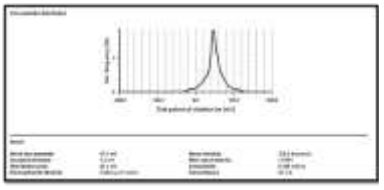
3.4.3 Zeta potential



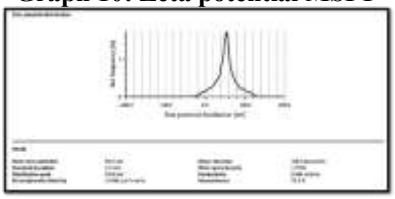
Graph 10: Zeta potential MSF1



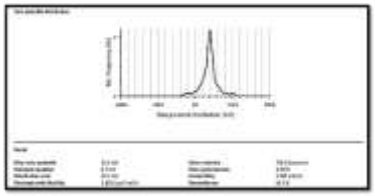
Graph 11: Zeta potential MSF2



Graph 12: Zeta potential MSF3



Graph 13: Zeta potential MSF4



Graph 14: Zeta potential MSF5

Graph 15: Graphical representation of Zeta potential

3.4.4 Scanning electron microscope (SEM)

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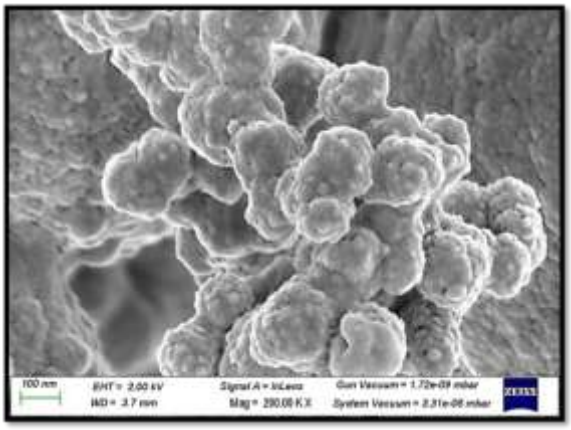
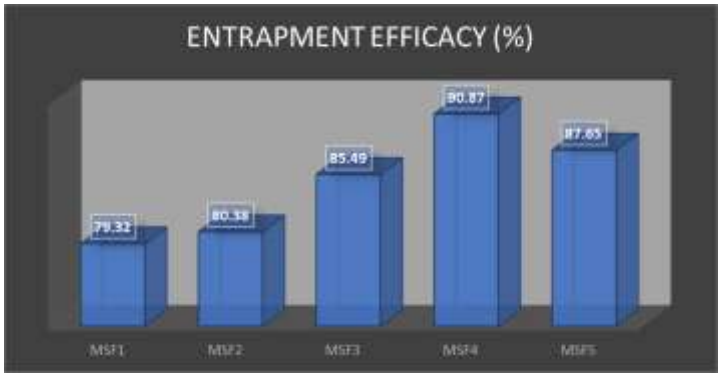


Figure 2: Scanning electron microscope (SEM)

3.5 Entrapment efficacy Determination of microsphere formulation

Table 7: Entrapment efficacy

Formulations	Entrapment efficacy (%)
MSF1	79.32
MSF2	80.38
MSF3	85.49
MSF4	**90.87**
MSF5	87.65



Graph 16: Graphical Representation of Entrapment efficacy determination

3.6 *In Vitro* drug release study of optimized microsphere

Table 8: *In-vitro* drug release studies

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
0.5	20.55	79.45	0.707	1.900	-0.301	1.313
2	35.46	64.54	1.414	1.810	0.301	1.550
3	42.3	57.7	1.732	1.761	0.477	1.626
5	54.38	45.62	2.236	1.659	0.699	1.735
7	66.49	33.51	2.646	1.525	0.845	1.823
8	75.51	24.49	2.828	1.389	0.903	1.878
10	82.45	17.55	3.162	1.244	1.000	1.916
13	91.37	8.63	3.606	0.936	1.114	1.961

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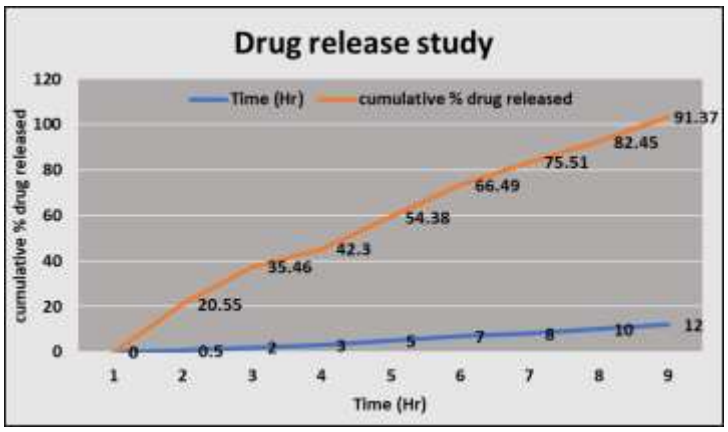
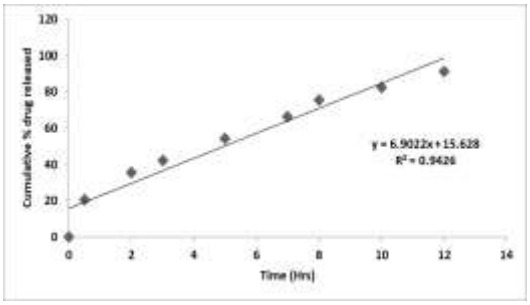


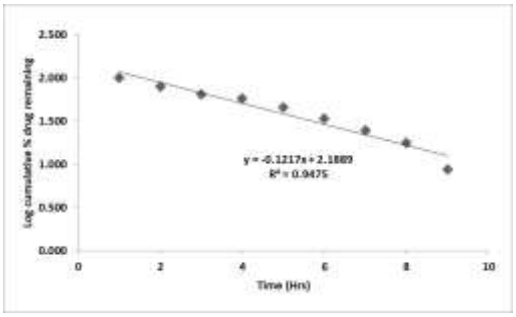
Graph 17: Drug release study

Table 9: Correlation value (R^2 value)

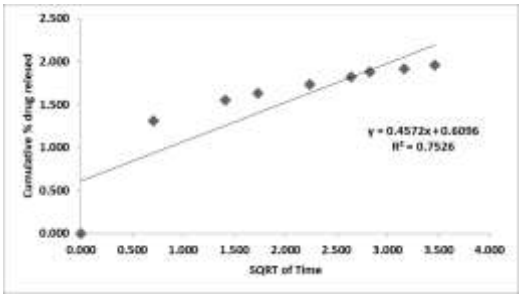
Formulation	Model	Kinetic parameter values
Microsphere F4 formulation	Zero Order	$R^2 = 0.942$
	First Order	$R^2 = 0.947$
	Higuchi	$R^2 = 0.752$
	Korsmeyer peppas	$R^2 = 0.506$



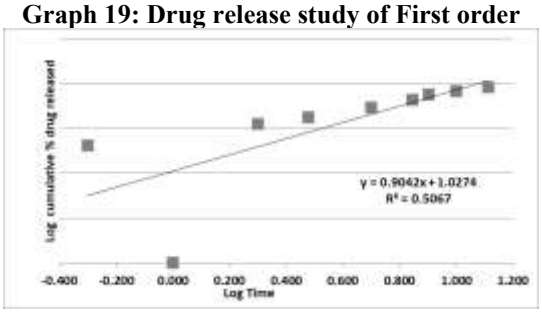
Graph 18: Drug release study of Zero order



Graph 19: Drug release study of First order



Graph 20: Drug release study of Higuchi order



Graph 21: Drug release study of Korsmeyer peppas

3.7 Results of antimicrobial activity of microsphere F4 formulation

3.7.1 Antimicrobial activity of Formulation against *Escherichia coli*

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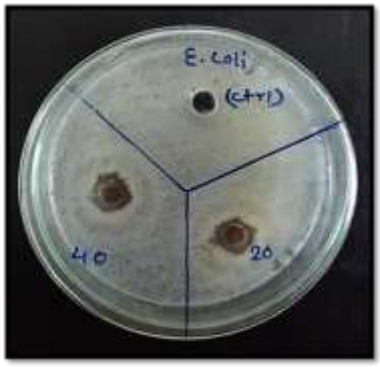
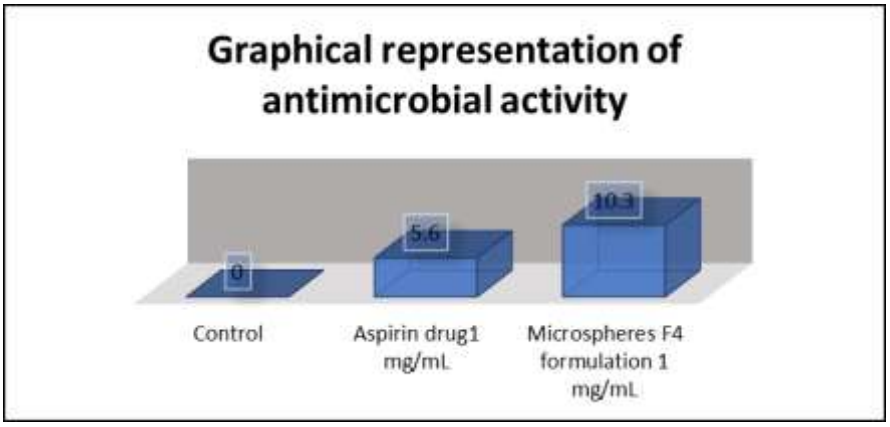


Figure 3: Antimicrobial activity

Table 10: Antimicrobial activity of Formulation against *Escherichia coli*

Sample Name	Zone of Inhibition (mm)
Control	0 mm
Ciprofloxacin drug1 mg/mL	5.6 mm
Microspheres F4 formulation 1 mg/mL	10.3 mm



Graph 22: Graphical representation of antimicrobial activity

Discussion

The preformulation studies of Ciprofloxacin confirmed its purity, stability, and suitability for microsphere formulation. UV and FTIR analyses verified the identity and structural integrity of the drug, while the calibration curve showed excellent linearity within the selected concentration range. Among all formulations, MSF4 exhibited the smallest particle size, highest zeta potential, and maximum entrapment efficiency, indicating better stability and drug incorporation. SEM analysis confirmed the formation of nearly spherical microspheres with suitable surface morphology. The optimized formulation showed sustained drug release over 13 hours following First-order kinetics and demonstrated enhanced antibacterial activity against Escherichia coli compared with pure drug solution. Overall, the developed Ciprofloxacin-loaded microspheres exhibited promising characteristics for controlled antimicrobial drug delivery.

4. CONCLUSION

The study successfully developed natural polymer–based Ciprofloxacin microspheres with optimized characteristics for sustained drug delivery and enhanced antimicrobial activity. The pre-formulation studies confirmed the purity, stability, and compatibility of Ciprofloxacin with the selected polymers. The optimized microsphere formulation (MSF4) exhibited desirable

physical characteristics, high entrapment efficiency, uniform particle size, and strong surface charge, ensuring stability and controlled release. *In-vitro* release studies demonstrated prolonged drug release, while antimicrobial evaluation confirmed superior efficacy compared to the pure drug. Overall, the results indicate that natural polymer–based microspheres are a promising platform for improving the therapeutic performance of Ciprofloxacin by providing controlled drug release, enhanced antibacterial activity, and potential for improved patient compliance. This formulation approach lays the foundation for further *in-vivo* studies and potential clinical applications in the treatment of bacterial infections.

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