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Journal of Drug Delivery and Therapeutics

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Research Article

# Formulation and Evaluation of Cefotaxime-Loaded PLGA Nanoparticles for the Treatment of Multidrug-Resistant Bacterial Infections

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## Article Info:

### Article History:

Received 12 April 2026
Reviewed 10 June 2026
Accepted 29 June 2026
Published 15 July 2026

### Cite this article as:

Sahu R, Khangar PK, Formulation and Evaluation of Cefotaxime-Loaded PLGA Nanoparticles for the Treatment of Multidrug-Resistant Bacterial Infections, Journal of Drug Delivery and Therapeutics. 2026; 16(7):59-69
*DOI: https://doi.org/10.22270/jddt.v16i7.7860*

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## Abstract

Multidrug-resistant (MDR) bacterial infections, particularly those caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, have substantially compromised the therapeutic efficacy of cefotaxime, a third-generation cephalosporin antibiotic. The present study was aimed at formulating and evaluating cefotaxime-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles as a controlled-release antibacterial delivery system capable of overcoming the pharmacokinetic and resistance-related limitations of the conventional drug. Nanoparticles were prepared by the solvent evaporation/nanoprecipitation technique, and formulation variables – namely drug-to-polymer ratio, PLGA concentration, surfactant (polyvinyl alcohol, PVA) concentration, stirring speed, and sonication time – were systematically optimized using a one-factor-at-a-time approach. The optimized formulation (drug:polymer 1:5, PLGA 2.0% w/v, PVA 1.5% w/v, stirring speed 14,000 rpm, sonication time 6 min) yielded nanoparticles with a mean particle size of 165.3 ± 1.9 nm, a polydispersity index (PDI) of 0.214 ± 0.02, and a zeta potential of −28.6 ± 1.4 mV. Drug loading and encapsulation efficiency were found to be 21.4 ± 0.3% and 93.8 ± 0.7%, respectively. Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) confirmed the absence of significant drug–polymer interaction. Scanning and transmission electron microscopy revealed smooth, spherical, well-dispersed nanoparticles. In vitro release studies demonstrated a biphasic pattern, with an initial burst release followed by sustained release of cefotaxime for up to 72 hours. Antibacterial evaluation by agar well diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time–kill assays revealed significantly enhanced and sustained activity of the nanoformulation against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae relative to free cefotaxime. The formulation remained physicochemically stable for 90 days under International Council for Harmonisation (ICH) long-term storage conditions and exhibited hemolysis below the 5% safety threshold, indicating excellent hemocompatibility. Collectively, these findings indicate that cefotaxime-loaded PLGA nanoparticles represent a promising, biocompatible, and scalable sustained-release platform with potential application in the treatment of MDR bacterial infections.

**Keywords:** Cefotaxime; PLGA nanoparticles; Multidrug resistance; Controlled drug release; Encapsulation efficiency; Antibacterial activity; Nanomedicine.

## 1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical threats to global public health, compromising the effective prevention and treatment of an ever-increasing range of bacterial infections. The World Health Organization has classified AMR among the ten greatest threats to global health, with projections suggesting that drug-resistant infections could claim up to 10 million lives annually by 2050 if current trends remain unaddressed.[1,2] Among the pathogens of greatest clinical concern are the so-called ESKAPE organisms – Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species – which collectively represent the principal cause of life-threatening nosocomial infections worldwide and exhibit alarmingly high levels of multidrug resistance (MDR).[3]

Cefotaxime, a third-generation cephalosporin β-lactam antibiotic, was historically considered a first-line therapeutic agent for serious Gram-negative infections, including meningitis, septicemia, pneumonia, and intra-abdominal infections. Its broad-spectrum bactericidal activity, achieved through inhibition of bacterial cell wall synthesis via penicillin-binding proteins (PBPs), made it an invaluable clinical tool.[4] However, the widespread emergence of extended-spectrum β-lactamase (ESBL)-producing organisms and carbapenem-resistant Enterobacteriaceae has severely compromised the therapeutic utility of cefotaxime, necessitating the development of innovative delivery strategies to restore and enhance its antimicrobial efficacy.[5,6] In addition, cefotaxime exhibits a short plasma elimination half-life of approximately 1.0–1.5 hours, necessitating frequent dosing and compromising the attainment of the time-dependent

pharmacokinetic–pharmacodynamic (PK-PD) index (T > MIC) most predictive of therapeutic outcome.[16,17,19]

Nanotechnology-based drug delivery systems have attracted substantial scientific and pharmaceutical interest as a promising strategy to overcome the limitations of conventional antibiotic formulations. In particular, polymeric nanoparticles fabricated from poly(lactic-co-glycolic acid) (PLGA) – a biodegradable, biocompatible, and FDA-approved polymer – offer compelling advantages for antibiotic delivery, including controlled and sustained drug release, protection of labile drugs from enzymatic degradation, enhanced intracellular penetration, and the potential to circumvent resistance mechanisms such as efflux pumps and biofilm formation.[7,8] By modulating particle size, surface charge, polymer molecular weight, and drug-to-polymer ratio, it is possible to engineer nanoparticulate formulations exhibiting superior drug loading efficiency, prolonged drug release profiles, and enhanced bactericidal activity compared with free drug solutions.[9,10]

Despite the considerable body of literature describing PLGA nanoparticles loaded with antibiotics such as gentamicin, cefuroxime, tobramycin, ciprofloxacin, and vancomycin, relatively few investigations have specifically addressed the encapsulation of cefotaxime – a drug of particular clinical importance for MDR Gram-negative infections – within PLGA nanoparticles.[39–42] Existing literature on cefotaxime nanoformulations is largely confined to chitosan-based or lipid-based carriers, and comprehensive physicochemical characterization combined with in vitro antibacterial assessment against MDR clinical isolates remains limited.[43,44] This knowledge gap provided the principal scientific impetus for the present investigation. Accordingly, the present study was designed to formulate cefotaxime-loaded PLGA nanoparticles using the double emulsion/solvent evaporation technique, optimize critical formulation and process variables, and comprehensively evaluate the resulting nanoparticulate system in terms of physicochemical characteristics, in vitro release kinetics, antibacterial activity against MDR clinical isolates, stability, and hemocompatibility.

## 2. Materials and Methods

### 2.1 Materials

Cefotaxime sodium, a third-generation cephalosporin antibiotic with broad-spectrum antibacterial activity, was used as the model drug owing to its established efficacy against both Gram-positive and Gram-negative bacteria, including MDR strains. Poly(lactic-co-glycolic acid) (PLGA) was selected as the polymeric carrier on account of its biodegradability, biocompatibility, controlled-release properties, and regulatory approval. Polyvinyl alcohol (PVA), Tween 80, and Poloxamer 188 were used as stabilizing surfactants. Dichloromethane, acetone, ethyl acetate, and methanol (all analytical grade) served as organic solvents. Phosphate buffer saline, hydrochloric acid, sodium hydroxide, potassium dihydrogen phosphate, distilled water, Mueller–Hinton broth, and Mueller–Hinton agar were of analytical grade. The antibacterial activity of the developed nanoparticles was evaluated against multidrug-resistant clinical isolates of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae procured from a recognized microbial culture collection centre.

### 2.2 Preformulation Studies

The organoleptic properties (colour, odour, taste, and physical form) of cefotaxime sodium were assessed visually and compared with standard reference characteristics. Solubility was determined in distilled water, phosphate buffer pH 7.4, methanol, ethanol, and acetone by adding an excess quantity of drug to each solvent, shaking for 24 hours at room temperature, filtering, and analyzing the filtrate spectrophotometrically. The absorption maximum (λmax) of cefotaxime sodium was determined in phosphate buffer pH 7.4 by scanning a suitable concentration of drug solution between 200 and 400 nm against the corresponding blank using a UV–visible spectrophotometer, performed in triplicate. A calibration curve was subsequently constructed using serial dilutions of cefotaxime sodium in phosphate buffer pH 7.4 and recording absorbance at λmax.

Drug–polymer compatibility was assessed by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). FTIR spectra of pure cefotaxime, PLGA, and their physical mixture were recorded between 4000 and 400 $cm^{-1}$ using the KBr pellet method and examined for evidence of chemical interaction. For DSC analysis, approximately 5–10 mg of each accurately weighed sample (cefotaxime, PLGA, and the physical mixture) was placed in sealed aluminium pans, with an empty pan used as reference, and heated from 25°C to 300°C at a rate of 10°C/min under a continuous nitrogen atmosphere. The resulting thermograms were evaluated for glass transition temperature (Tg), melting endotherms (Tm), and any shifts indicative of drug–polymer interaction.

### 2.3 Optimization of Formulation Variables

Formulation and process variables were optimized using a one-factor-at-a-time approach to obtain cefotaxime-loaded PLGA nanoparticles with desirable physicochemical characteristics and sustained drug release behaviour. The variables investigated included drug-to-polymer ratio, PLGA concentration, surfactant (PVA) concentration, stirring speed, and sonication time. Each parameter was varied systematically while keeping the remaining variables constant, and the resulting formulations were evaluated for particle size, entrapment efficiency, drug loading, and in vitro drug release.

### 2.4 Preparation of Cefotaxime-Loaded PLGA Nanoparticles

Cefotaxime-loaded PLGA nanoparticles were prepared by the solvent evaporation/nanoprecipitation method owing to its simplicity, reproducibility, and suitability for producing polymeric nanoparticles with controlled

particle size and sustained drug release. Briefly, PLGA was accurately weighed and dissolved in a suitable organic solvent (dichloromethane or acetone) under continuous stirring until a clear, homogeneous polymer solution was obtained. Cefotaxime sodium was then incorporated into the polymeric solution under continuous stirring to ensure uniform drug dispersion within the polymer matrix. The resulting drug–polymer solution was added dropwise into an aqueous phase containing PVA as stabilizer under constant magnetic stirring to form a stable emulsion. The emulsion was subjected to probe sonication for a predetermined period to reduce droplet size and obtain nanoparticles with a narrow size distribution. The organic solvent was subsequently evaporated under continuous magnetic stirring at room temperature, leading to nanoparticle hardening through polymer precipitation. The formed nanoparticles were recovered by high-speed centrifugation, washed repeatedly with distilled water to remove residual solvent, unentrapped drug, and excess surfactant, and finally freeze-dried to obtain a dry, free-flowing nanoparticulate powder, which was stored in airtight containers under suitable conditions until further evaluation.

### 2.5 Physicochemical Characterization

Particle size, polydispersity index (PDI), and zeta potential of the optimized formulation were determined by dynamic light scattering (DLS) after dispersing the nanoparticles in distilled water. Surface morphology was examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM); for SEM, nanoparticle samples were mounted on metallic stubs, while for TEM, diluted nanoparticle suspensions were placed on carbon-coated copper grids.

Drug loading was determined by dissolving an accurately weighed quantity of nanoparticles in a suitable solvent system to completely extract the entrapped drug, followed by filtration and spectrophotometric analysis at the predetermined wavelength using the previously constructed calibration curve. Encapsulation efficiency was determined by the indirect method, in which the nanoparticle suspension was centrifuged at high speed and the supernatant containing unentrapped free drug was analyzed spectrophotometrically. Drug loading and encapsulation efficiency were calculated using the following equations:

$$\text{Drug loading (\%)} = \left(\frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}}\right) \times 100$$

$$\text{Encapsulation efficiency (\%)} = \left(\frac{\text{Total drug added} - \text{Free drug in supernatant}}{\text{Total drug added}}\right) \times 100$$

### 2.6 In Vitro Drug Release Studies

In vitro release of cefotaxime from the optimized PLGA nanoparticles was evaluated using the dialysis bag diffusion technique. An accurately weighed quantity of nanoparticles equivalent to a known amount of cefotaxime was dispersed in a small volume of dissolution medium and placed inside a pre-soaked dialysis membrane, securely tied at both ends. The dialysis bag was immersed in phosphate buffer pH 7.4 maintained at 37 ± 0.5°C under continuous magnetic stirring to simulate physiological conditions. At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions, and the withdrawn samples were analyzed spectrophotometrically at the predetermined wavelength of cefotaxime.

### 2.7 Antibacterial Activity Evaluation

The antibacterial efficacy of pure cefotaxime solution, blank PLGA nanoparticles, and cefotaxime-loaded PLGA nanoparticles was evaluated against MDR clinical isolates of E. coli, S. aureus, P. aeruginosa, and K. pneumoniae using the agar well diffusion method. Mueller–Hinton agar plates were uniformly inoculated with standardized bacterial cultures using sterile cotton swabs, wells were prepared using a sterile cork borer, and the test samples were introduced into the wells under aseptic conditions. Plates were incubated at 37°C for 24 hours, after which the diameter of the zone of inhibition was measured using a calibrated scale.

The minimum inhibitory concentration (MIC) was determined by the broth dilution method, in which serial concentrations of the nanoparticle formulation were prepared in sterile nutrient broth, inoculated with standardized bacterial suspensions, and incubated at 37°C for 24 hours; the lowest concentration showing no visible turbidity was recorded as the MIC. The minimum bactericidal concentration (MBC) was determined by subculturing samples from MIC tubes showing no visible growth onto sterile agar plates, with the lowest concentration showing complete absence of bacterial colonies recorded as the MBC. Time–kill assays were performed by treating bacterial cultures with the optimized nanoparticle formulation, withdrawing samples at predetermined time intervals, and determining viable bacterial counts to assess the rate and extent of bacterial killing over time.

### 2.8 Stability Studies

Stability studies were conducted in accordance with International Council for Harmonisation (ICH) guidelines under long-term (25 ± 2°C / 60 ± 5% relative humidity) and accelerated (40 ± 2°C / 75 ± 5% relative humidity) storage conditions for a period of three months. Samples were withdrawn at predetermined time intervals and evaluated for particle size, drug content, entrapment efficiency, zeta potential, and physical appearance.

### 2.9 Hemolysis (Hemocompatibility) Assay

The hemolytic toxicity of cefotaxime-loaded PLGA nanoparticles was evaluated using freshly collected

human red blood cells (RBCs). Erythrocytes were separated by centrifugation, washed repeatedly with isotonic phosphate-buffered saline, and incubated with different concentrations of the nanoparticle formulation at 37°C for a specified period. Distilled water and PBS served as positive and negative controls, respectively, representing complete and negligible hemolysis. Following incubation, samples were centrifuged and the supernatant was analyzed spectrophotometrically for released hemoglobin, and the percentage hemolysis was calculated relative to the positive control.

## 3. Results

### 3.1 Preformulation Studies

Cefotaxime sodium appeared as a white to off-white crystalline powder, odourless, with a slightly bitter taste, consistent with standard reference characteristics. The drug was found to be freely soluble in distilled water and phosphate buffer pH 7.4, soluble in methanol, slightly soluble in ethanol, and practically insoluble in acetone (Table 1), confirming its suitability for incorporation into PLGA nanoparticles by the nanoprecipitation/solvent evaporation technique. The λmax of cefotaxime sodium in phosphate buffer pH 7.4 was found to be 235 nm, and the calibration curve constructed over the concentration range of 2–12 µg/mL showed excellent linearity, with a correlation coefficient ($R^2$) of 0.999.

**Table 1:** Solubility profile of cefotaxime sodium in various solvents.

| Solvent | Solubility |
|---|---|
| Distilled water | Freely soluble |
| Phosphate buffer pH 7.4 | Freely soluble |
| Methanol | Soluble |
| Ethanol | Slightly soluble |
| Acetone | Practically insoluble |

### 3.2 Drug–Polymer Compatibility Studies

FTIR spectra of pure cefotaxime sodium, PLGA, and their physical mixture exhibited all characteristic peaks of the individual components without the appearance of new peaks or major peak shifts, indicating the absence of any significant chemical interaction between drug and polymer.

DSC analysis revealed that pure cefotaxime exhibited a sharp melting endotherm at approximately 171.2°C, confirming its crystalline nature, while PLGA exhibited a glass transition temperature (Tg) of approximately 52.3°C and a broad melting peak around 159.8°C, characteristic of its amorphous nature. In the physical mixture, both characteristic peaks were retained with minor shifts (Tg of PLGA at ~51.4°C; cefotaxime melting peak at ~156.5°C) and reduced enthalpy, with no new peaks or disappearance of existing peaks, indicating good thermal compatibility between the drug and polymer.

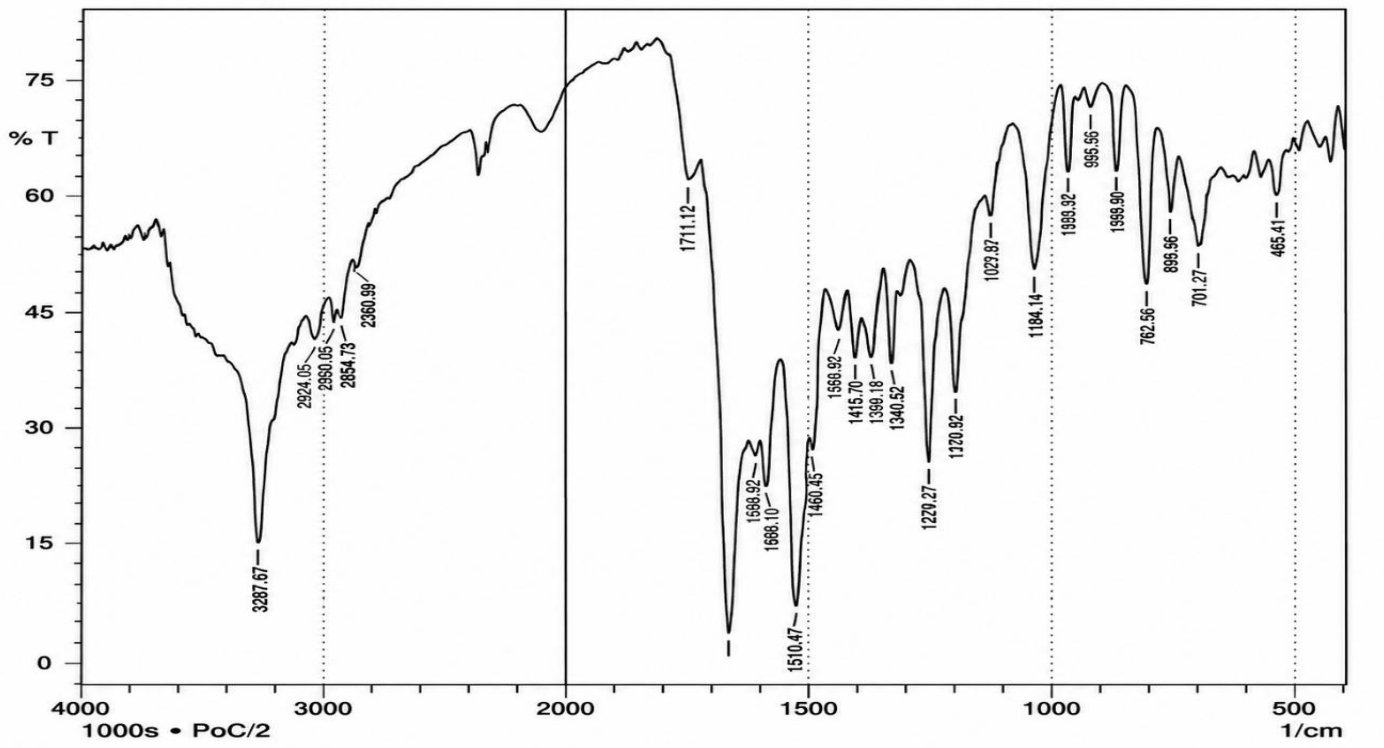



*Figure 1: FTIR spectrum of pure cefotaxime sodium.*

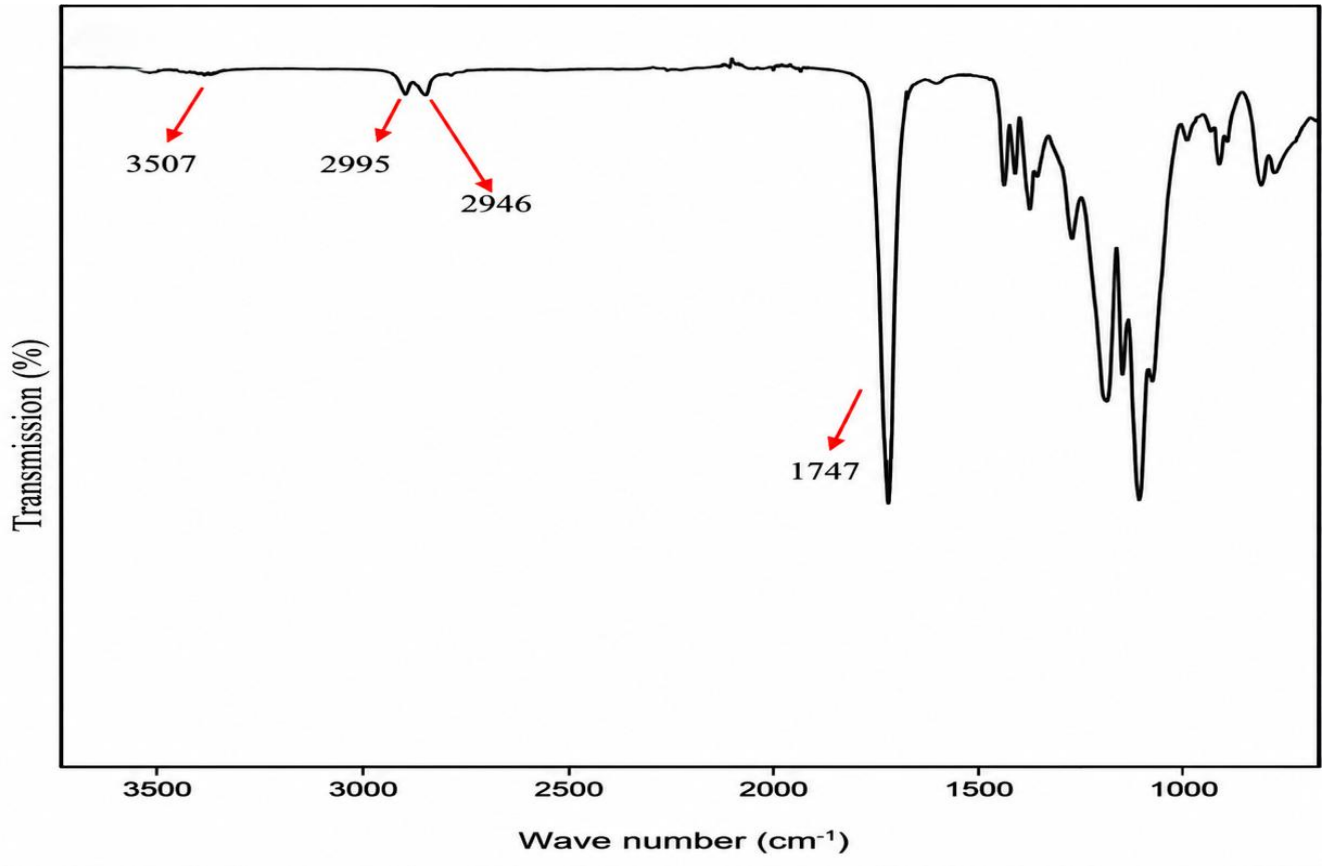



*Figure 2: FTIR spectrum of PLGA.*

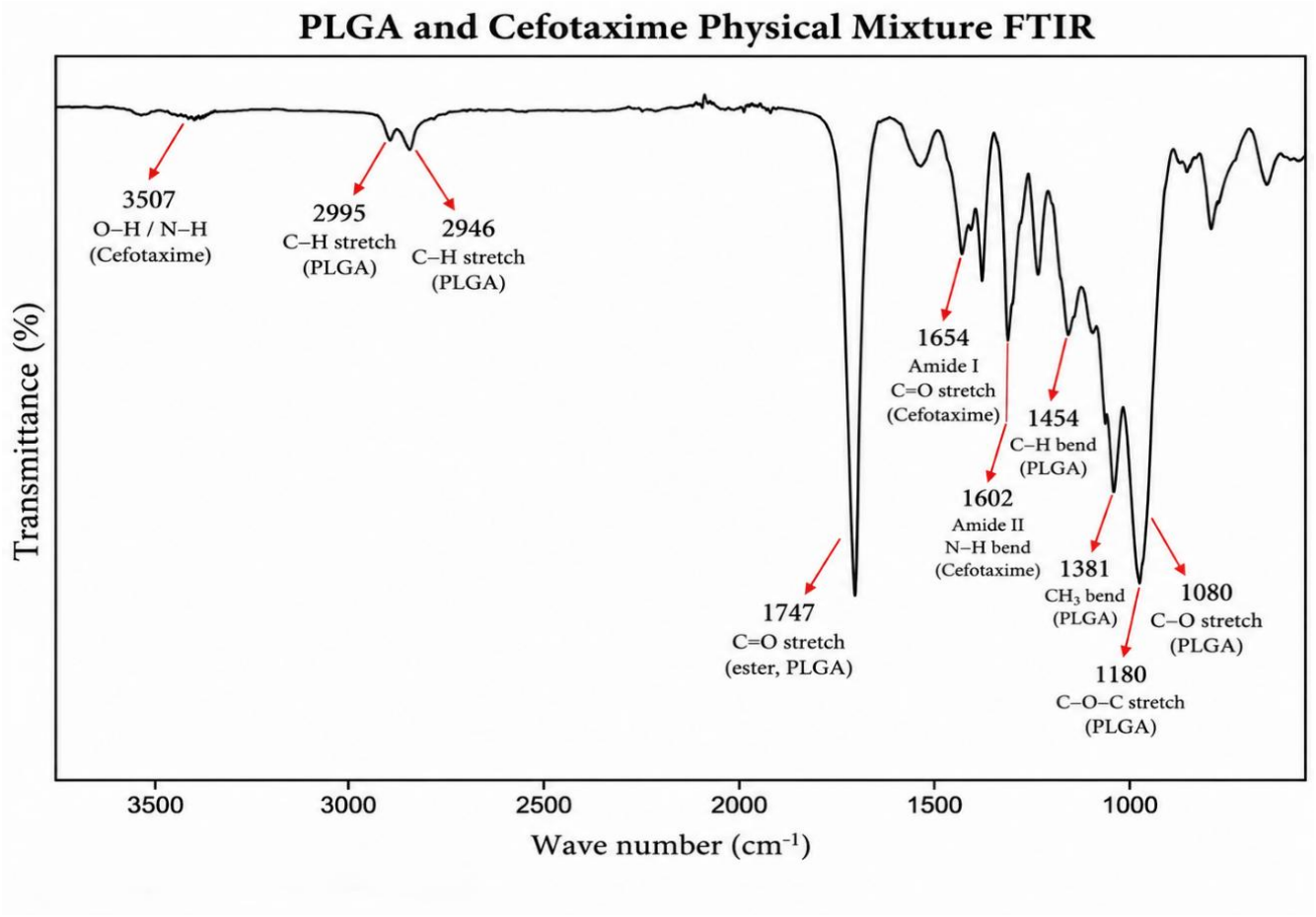
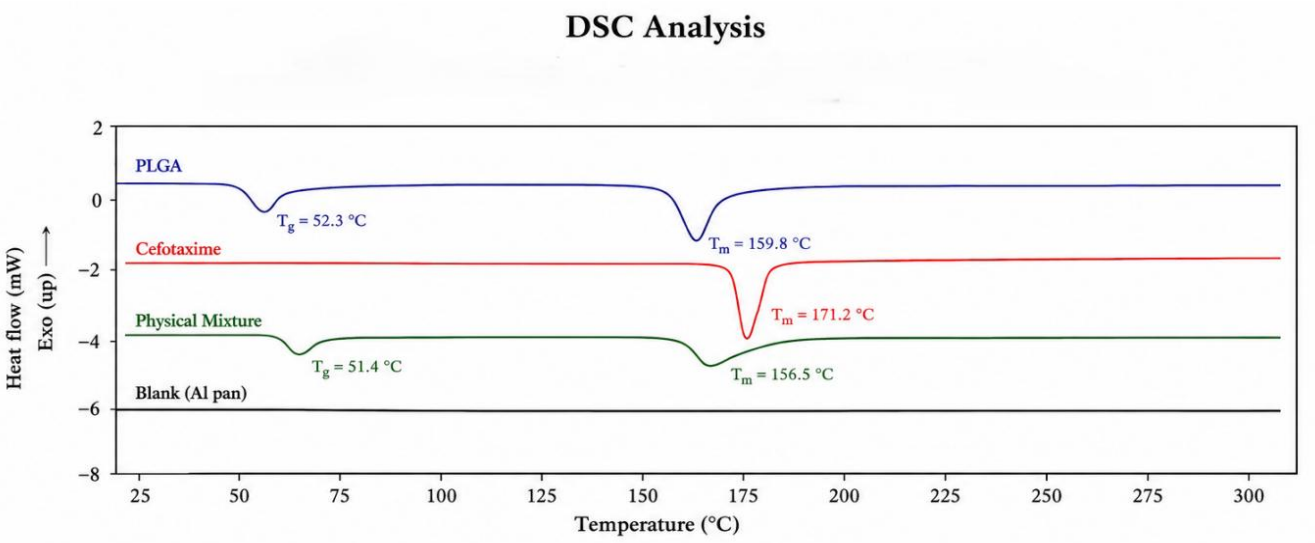



*Figure 3. FTIR spectrum of the PLGA–cefotaxime physical mixture, showing retention of characteristic peaks of both components without appearance of new peaks, indicating drug–polymer compatibility.*




| Sample | Glass Transition ($T_g$) | | Melting Endotherm ($T_m$) | | | Observation |
|---|---|---|---|---|---|---|
| | Onset (°C) | Midpoint (°C) | Onset (°C) | Peak (°C) | Enthalpy ($\Delta H$, J/g) | |
| PLGA | 46.8 | 52.3 | 153.2 | 159.8 | 45.6 | Shows $T_g$ and melting endotherm characteristic of PLGA. |
| Cefotaxime | – | – | 166.3 | 171.2 | 78.9 | Sharp melting endotherm characteristic of cefotaxime. |
| Physical Mixture | 45.7 | 51.4 | 150.8 | 156.5 | 32.1 | $T_g$ of PLGA retained; melting peak of cefotaxime reduced and slightly shifted. |
| Blank (Al pan) | – | – | – | – | – | No thermal events observed. |

*Note: $T_g$ = Glass transition temperature; $T_m$ = Melting temperature; $\Delta H$ = Enthalpy of fusion.*

*Figure 4. DSC thermograms of PLGA, cefotaxime, the physical mixture, and blank aluminium pan, showing retention of characteristic glass transition (Tg) and melting endotherm (Tm) peaks with minor shifts in the physical mixture.*

### *3.3 Optimization of Formulation Variables*

Increasing the polymer concentration enhanced entrapment efficiency owing to formation of a thicker polymeric matrix around the drug, although excessively high polymer concentrations produced larger particles due to increased viscosity of the organic phase. Increasing PLGA concentration similarly improved encapsulation efficiency and prolonged drug release, while lower concentrations resulted in poor encapsulation and rapid release. Increasing surfactant (PVA) concentration reduced particle size by lowering interfacial tension between phases, although very high surfactant levels slightly reduced entrapment efficiency due to enhanced drug diffusion into the external aqueous phase. Higher stirring speed produced smaller emulsion droplets and reduced nanoparticle size, whereas extremely high stirring speeds caused emulsion instability and possible drug leakage. Increasing sonication time initially decreased particle size by efficient droplet breakdown, but excessive sonication generated heat that could compromise drug stability and reduce encapsulation efficiency.

Based on the desirability approach generated using Design-Expert software, batch F5 (drug:polymer ratio 1:5, PLGA 2.0% w/v, PVA 1.5% w/v, stirring speed 14,000 rpm, sonication time 6 minutes) exhibited the most desirable characteristics among all formulations, with minimum particle size, high entrapment efficiency, satisfactory drug loading, and sustained drug release behaviour, and was therefore selected as the optimized formulation for further evaluation.

### *3.4 Physicochemical Characterization of Optimized Nanoparticles*

**Table :.** *Physicochemical characteristics of the optimized cefotaxime-loaded PLGA nanoparticles.*

| Parameter | Value |
|---|---|
| Mean particle size | 165.3 ± 1.9 nm |
| Polydispersity index (PDI) | 0.214 ± 0.02 |
| Zeta potential | −28.6 ± 1.4 mV |
| Drug loading | 21.4 ± 0.3% |
| Encapsulation efficiency | 93.8 ± 0.7% |

The optimized formulation exhibited a mean particle size of 165.3 ± 1.9 nm, well within the nanometric range suitable for controlled drug delivery, attributable to efficient emulsification and sonication during preparation. The low PDI (0.214 ± 0.02) indicated a narrow particle size distribution and good homogeneity, while the zeta potential of −28.6 ± 1.4 mV reflected sufficient electrostatic repulsion to confer good physical stability, with the negative surface charge attributed mainly to PLGA. SEM and TEM analyses revealed predominantly spherical nanoparticles with smooth, uniform surface morphology, minimal aggregation, and a dense, homogeneous internal structure, with particle sizes

correlating well with DLS measurements. The optimized formulation displayed a drug loading of 21.4 ± 0.3% and an encapsulation efficiency of 93.8 ± 0.7%, reflecting efficient drug incorporation attributable to the optimized drug-to-polymer ratio, effective emulsification, and rapid nanoparticle solidification during solvent evaporation.

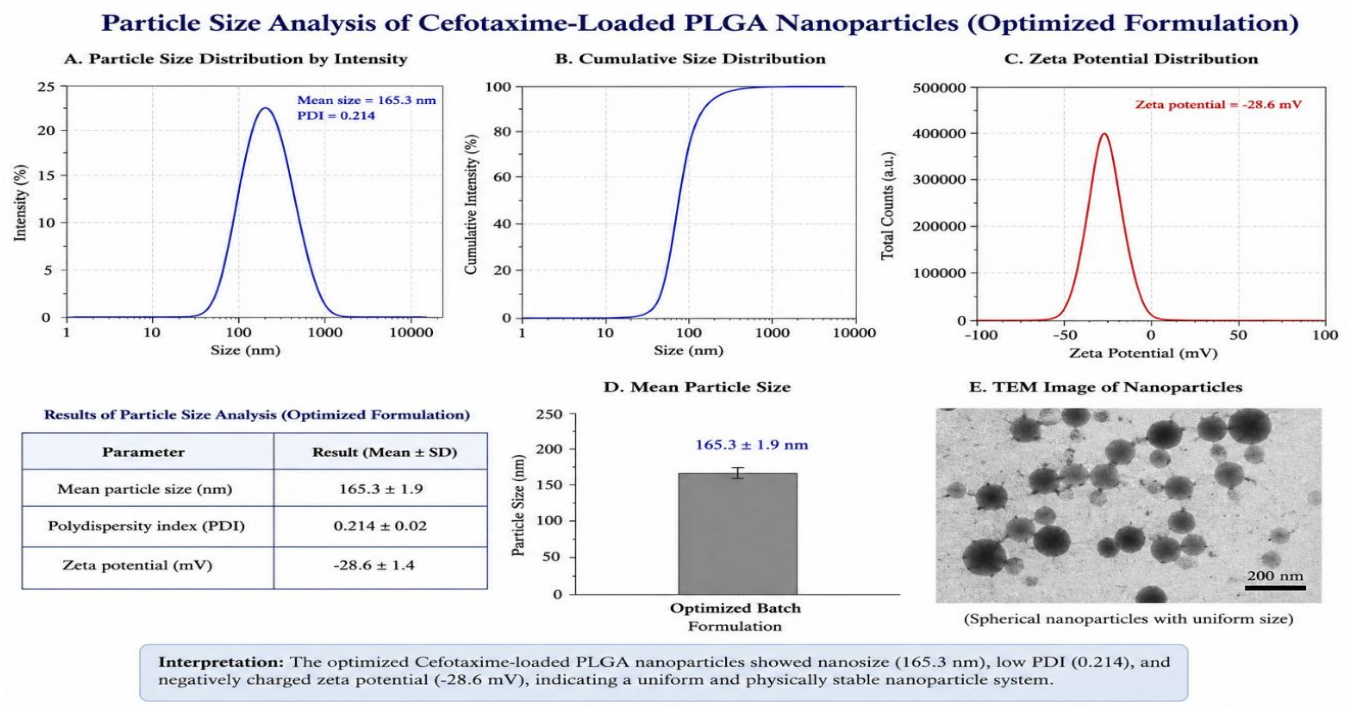



*Figure 5. Particle size distribution by intensity, cumulative size distribution, zeta potential distribution, mean particle s ize, and TEM image of the optimized cefotaxime-loaded PLGA nanoparticles, showing spherical nanoparticles of uniform size (~165.3 ± 1.9 nm), low PDI (0.214), and negative zeta potential (−28.6 mV).*

### *3.5 In Vitro Drug Release*

The optimized cefotaxime-loaded PLGA nanoparticles exhibited a biphasic release profile, characterized by an initial burst release attributable to surface-associated drug, followed by a prolonged and gradual release phase extending up to 72 hours. This sustained release behaviour was attributed to slow diffusion of the drug through the PLGA matrix coupled with gradual polymer erosion, indicating suitability of the developed system for controlled drug delivery with prolonged therapeutic effect.

### *3.6 Antibacterial Activity*

The optimized cefotaxime-loaded PLGA nanoparticles exhibited significant antibacterial activity against all tested microorganisms, producing comparatively larger and more sustained zones of inhibition than free cefotaxime solution, while blank PLGA nanoparticles showed no significant antibacterial activity, confirming that the observed effect was drug-mediated. The highest antibacterial activity was observed against E. coli and S. aureus, with zones of inhibition of 28.4 ± 1.2 mm and 26.7 ± 1.1 mm, respectively, while moderate activity was observed against P. aeruginosa and K. pneumoniae (Table 3).

**Table 3:** *Comparative antibacterial activity (zone of inhibition) of free cefotaxime and cefotaxime -loaded PLGA nanoparticles (CTX-PLGA NPs) against MDR bacterial strains.*

| Bacterial strain | Zone of inhibition – free cefotaxime (mm) | Zone of inhibition – CTX-PLGA NPs (mm) |
|---|---|---|
| Escherichia coli | Smaller | 28.4 ± 1.2 |
| Staphylococcus aureus | Smaller | 26.7 ± 1.1 |
| Pseudomonas aeruginosa | Smaller | Moderate activity |
| Klebsiella pneumoniae | Smaller | Moderate activity |

The optimized nanoparticle formulation exhibited lower MIC and MBC values against all tested strains compared with conventional cefotaxime sodium solution, indicating enhanced antibacterial potency and improved drug availability attributable to sustained release and improved nanoparticle–bacterial cell interactions. Time–kill assays demonstrated a progressive reduction in bacterial population over time, with significant bacterial killing observed within the first 6 hours and near-complete eradication of E. coli after 24 hours, confirming sustained bactericidal activity of the nanoformulation.

Overall, the comparative antibacterial study demonstrated that the developed cefotaxime-loaded PLGA nanoparticles possessed superior antibacterial efficacy – evidenced by larger zones of inhibition, lower MIC and MBC values, and prolonged bactericidal action – compared with the conventional cefotaxime formulation, attributable to improved drug encapsulation, sustained release, prolonged retention at the site of action, and enhanced nanoparticle penetration into bacterial cells.

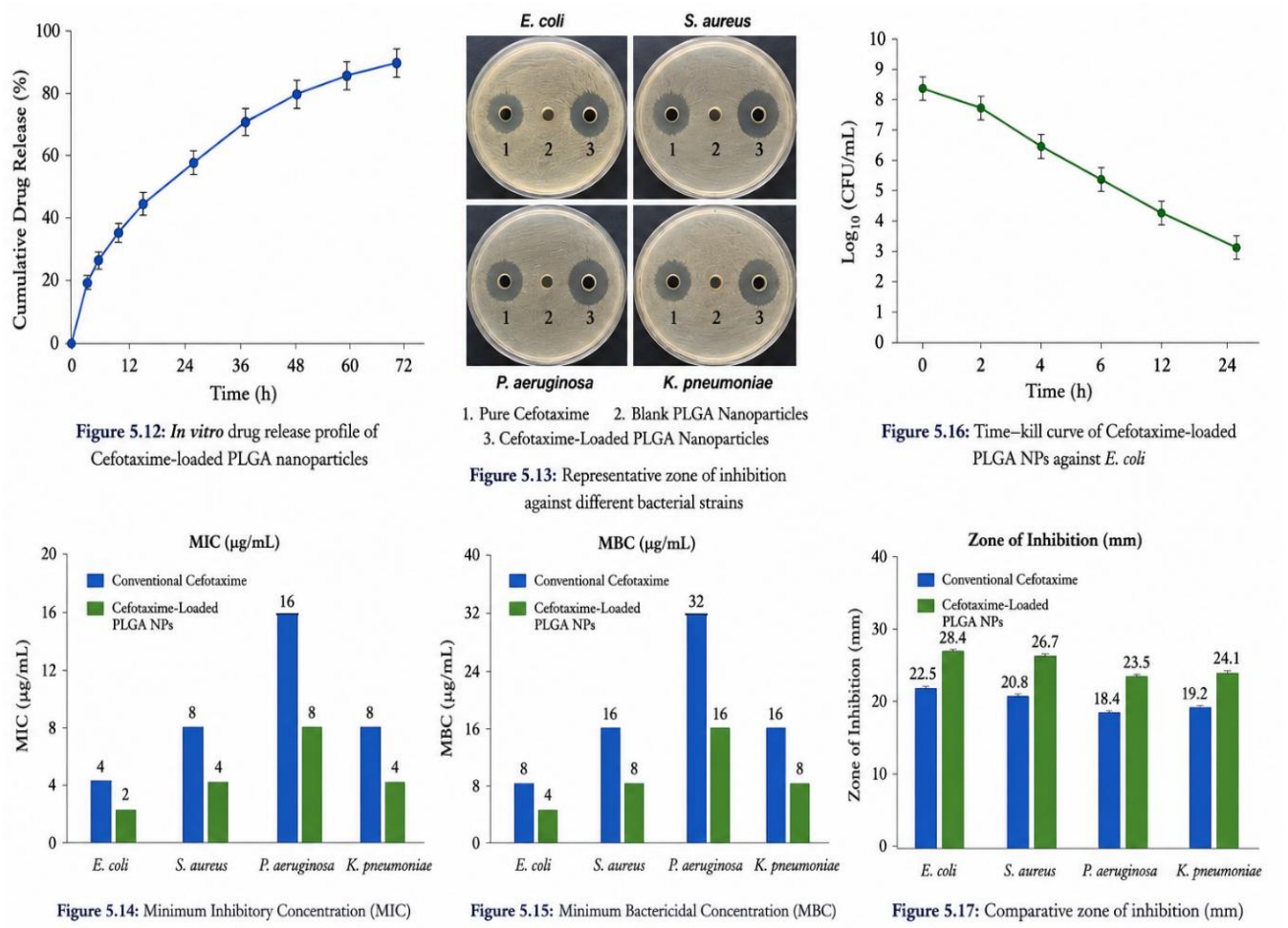



**Figure 5.12:** *In vitro* drug release profile of Cefotaxime-loaded PLGA nanoparticles

**Figure 5.13:** Representative zone of inhibition against different bacterial strains

**Figure 5.16:** Time–kill curve of Cefotaxime-loaded PLGA NPs against *E. coli*

**Figure 5.14:** Minimum Inhibitory Concentration (MIC)

**Figure 5.15:** Minimum Bactericidal Concentration (MBC)

**Figure 5.17:** Comparative zone of inhibition (mm)

*Figure 6. In vitro drug release profile, representative zones of inhibition against E. coli, S. aureus, P. aeruginosa, and K. pneumoniae, time–kill curve against E. coli, and comparative MIC, MBC, and zone-of-inhibition values for conventional cefotaxime versus cefotaxime-loaded PLGA nanoparticles.*

### *3.7 Stability Studies*

Under long-term storage conditions (25 ± 2°C / 60 ± 5% RH), the optimized formulation exhibited negligible changes in all evaluated parameters over 90 days. Particle size increased only marginally, from 165.3 ± 1.9 nm to 171.4 ± 2.3 nm, while drug content and entrapment efficiency remained above 90% and zeta potential values remained sufficiently negative, indicating maintenance of colloidal stability. Under accelerated storage conditions (40 ± 2°C / 75 ± 5% RH), comparatively greater but still acceptable variations were observed, including a moderate increase in particle size and slight reductions in drug content and entrapment efficiency, without significant aggregation or phase separation.

### *3.8 Hemocompatibility*

The optimized cefotaxime-loaded PLGA nanoparticles exhibited very low hemolytic activity at all tested concentrations, remaining below the acceptable safety limit of 5%, indicating that the nanoparticles did not cause significant damage to red blood cell membranes. This favourable hemocompatibility profile was attributed to the biocompatible and biodegradable nature of PLGA together with the smooth surface morphology and stable physicochemical properties of the nanoparticles.

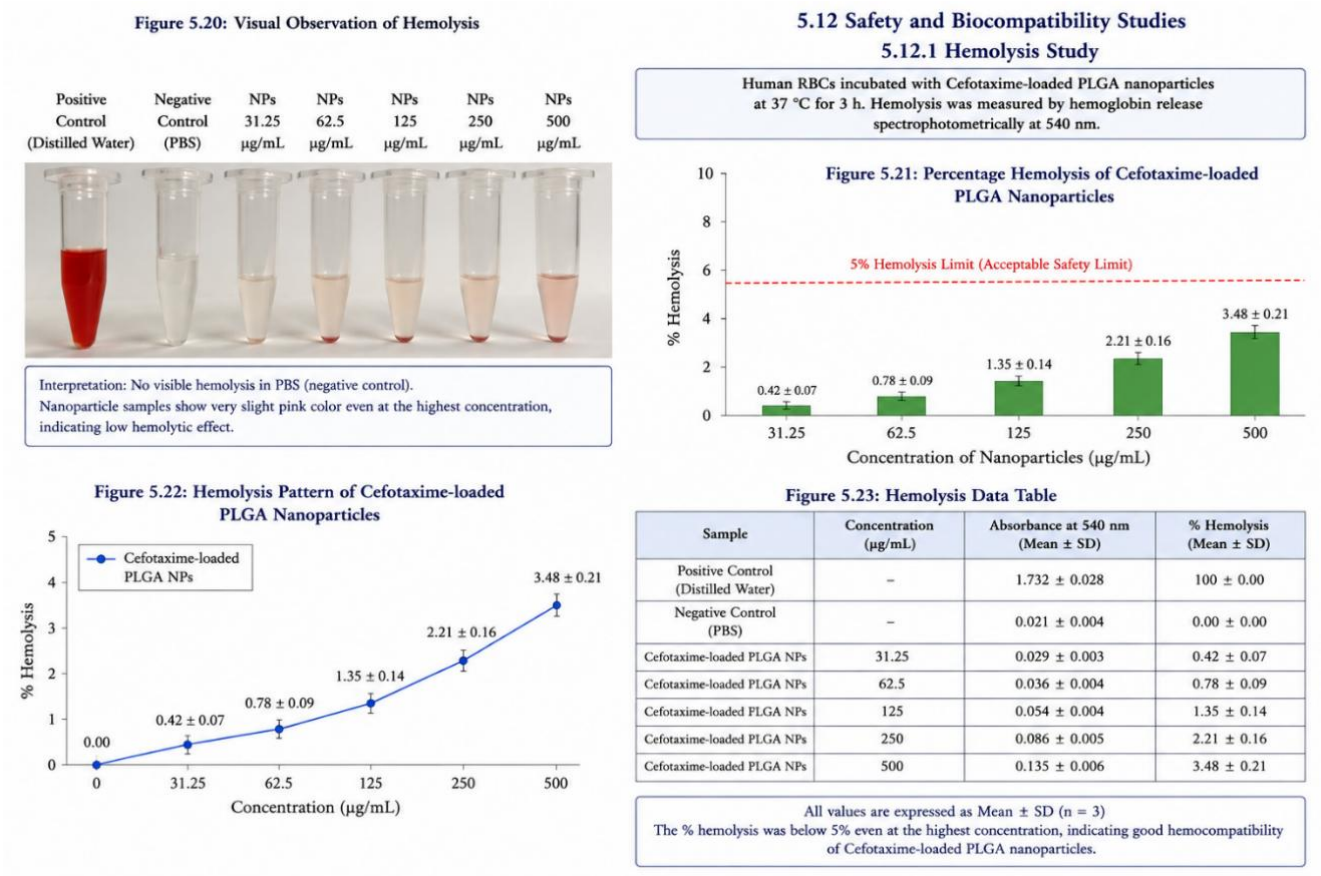



*Figure 7. Visual observation of hemolysis, percentage hemolysis of cefotaxime-loaded PLGA nanoparticles at increasing concentrations (31.25–500 µg/mL), hemolysis pattern, and hemolysis data table, showing %hemolysis well below the 5% safety limit at all tested concentrations.*

## 4. Discussion

The present study demonstrates the successful formulation of cefotaxime-loaded PLGA nanoparticles possessing physicochemical attributes consistent with the predefined hypothesis – namely a particle size below 300 nm, a PDI below 0.3, a zeta potential exceeding ±25 mV in magnitude, and an encapsulation efficiency above 70%. The compatibility studies (FTIR and DSC) confirmed that cefotaxime and PLGA can be co-formulated without significant chemical interaction, supporting the chemical stability of the active drug within the polymeric matrix. The systematic optimization of formulation and process variables – drug-to-polymer ratio, polymer concentration, surfactant concentration, stirring speed, and sonication time – revealed clear and mechanistically interpretable trends consistent with previously reported PLGA nanoparticle systems, in which polymer concentration governs matrix density and entrapment, while surfactant concentration and energy input (stirring and sonication) primarily govern droplet and particle size.

The nanoscale dimensions, narrow size distribution, and sufficiently negative zeta potential of the optimized formulation indicate good colloidal stability and suitability for parenteral administration, while particles in the 100–300 nm range are reported to favour macrophage uptake and lymphatic trafficking, properties of particular relevance for intracellular bacterial infections.

The biphasic in vitro release profile, comprising an initial burst followed by sustained release over 72 hours, is characteristic of matrix-type PLGA systems and is governed by a combination of diffusion of surface-associated drug and gradual polymer erosion.[26] Such a release profile is mechanistically well suited to overcoming the principal pharmacokinetic limitation of conventional cefotaxime – its short plasma half-life – by maintaining drug concentrations above the minimum inhibitory concentration for extended periods, thereby optimizing the time-dependent PK-PD index (T > MIC) that governs cephalosporin efficacy.[19]

The markedly enhanced antibacterial activity of the nanoformulation – evidenced by larger zones of inhibition, lower MIC and MBC values, and more rapid and sustained bactericidal action in time–kill assays – relative to free cefotaxime is consistent with the proposed mechanisms by which PLGA nanoparticles enhance antibiotic performance, including protection of the encapsulated drug from enzymatic (β-lactamase-mediated) degradation, sustained release maintaining therapeutic concentrations, and improved nanoparticle–bacterial cell interactions facilitating drug entry via endocytotic pathways that may bypass efflux pump-mediated resistance. The absence of antibacterial activity for blank PLGA nanoparticles confirms that the observed effect is attributable specifically to cefotaxime, ruling out an intrinsic antibacterial contribution from the polymer itself.

These findings are broadly consistent with earlier reports describing enhanced antibacterial performance of PLGA-encapsulated cephalosporins, aminoglycosides, and glycopeptides against resistant organisms, and extend this body of evidence specifically to cefotaxime, for which nanoparticulate formulations against MDR clinical isolates have previously been only sparsely characterized.

The satisfactory stability of the optimized formulation under ICH long-term storage conditions, together with hemolysis values well below the 5% safety threshold, support the pharmaceutical and translational feasibility of the developed nanoparticulate system. Taken together, the high encapsulation efficiency, favourable nanoscale characteristics, sustained drug release, enhanced antibacterial activity against MDR strains, satisfactory stability, and excellent hemocompatibility collectively address the principal limitations of conventional cefotaxime therapy – namely short half-life, frequent dosing, enzymatic degradation, and reduced efficacy against resistant organisms – and support the potential of this nanoformulation as an advanced delivery platform for MDR bacterial infections.

Limitations of the present study include its reliance on in vitro evaluation; further in vivo pharmacokinetic, pharmacodynamic, and toxicological studies in suitable animal infection models will be required to substantiate the translational potential of this formulation. Future work should also address anti-biofilm efficacy, intracellular antibacterial activity in macrophage infection models, and scale-up feasibility under quality-by-design frameworks.

## 5. Conclusion

Cefotaxime-loaded PLGA nanoparticles were successfully developed using the solvent evaporation/nanoprecipitation technique, with the optimized formulation exhibiting a mean particle size of 165.3 ± 1.9 nm, PDI of 0.214 ± 0.02, zeta potential of −28.6 ± 1.4 mV, drug loading of 21.4 ± 0.3%, and encapsulation efficiency of 93.8 ± 0.7%. The nanoformulation demonstrated sustained drug release over 72 hours, significantly enhanced antibacterial activity against MDR strains of E. coli, S. aureus, P. aeruginosa, and K. pneumoniae compared with free cefotaxime, satisfactory stability under ICH-recommended long-term storage conditions, and excellent hemocompatibility. These findings collectively support cefotaxime-loaded PLGA nanoparticles as a promising, biocompatible, sustained-release strategy for restoring and enhancing the therapeutic efficacy of cefotaxime against multidrug-resistant bacterial infections, warranting further in vivo evaluation.

**Conflict of Interest:** The authors declare no conflict of interest.

**Acknowledgements:** The authors acknowledge the Department of Pharmaceutical Sciences, Mangalayatan University, Jabalpur, for providing the necessary facilities to carry out this research work.

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