Natural Resources for Human Health

eISSN: 2583-1194

Original Research

Received 20 May 2026
Revised 28 June 2026
Accepted 20 July 2026

Natural Resources for Human Health 2026, Vol. 6 (8s): 205–220

KEYWORDS:
Curcumin, Quinazoline, Polymeric nanoparticles, Antibacterial therapy, Multidrug-resistant bacteria, Molecular hybridization, HPLC, LC-MS, Drug-release kinetics, Reactive oxygen species (ROS), Minimum inhibitory concentration (MIC), Biofilm inhibition, Nanomedicine.

Design and Development of Curcumin-Derived Quinazoline Hybrid Molecules and their Polymeric Nanoparticles for Targeted Antibacterial Therapy: Physicochemical Characterization, HPLC/LC-MS Quantification, Drug-Release Kinetics, ROS-Mediated Mechanistic Studies, MIC/MBC, Biofilm Inhibition and In Vitro Pharmacological Evaluation

Haragouri Mishra[1], Ajab Singh Choudhary[2], A Sree Geetha[3], Mahalakshmi Kodadi[4], Bhagwati Devi[5], Khushboo Majumdar[6], Shilpi Prasad[7], Fathima Grace Xavier[8], Varaganti Sai Chitra Prathyusha[9*]

[1]Assistant Professor, School of Pharmacy, Centurion University of Technology and Management, Odisha, India
[2]Assistant Professor, Medical Laboratory Technology, School of Allied Health Sciences (SOAHS), Noida International University, 17A Yamuna Expressway, Greater Noida, Uttar Pradesh, India
[3]Associate Professor, Department of Pharmaceutical Analysis, Aditya College of Pharmacy, Surampalem–533437, Andhra Pradesh, India
[4]Professor & Deputy Director, Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University Chhattisgarh, Kharora, Raipur, Chhattisgarh, India
[5]Associate Professor, School of Pharmaceutical Sciences, CGC University, Mohali–140307, Punjab, India
[6]Assistant Professor, Sahu Onkar Saran School of Pharmacy, Faculty of Pharmacy, IFTM University, Moradabad–244102, Uttar Pradesh, India
[7]Professor, Department of Pharmaceutics, Siddhi Vinayaka Institute of Technology and Sciences, Near Deendayal Awas Yojna, Mangla, Bilaspur, Chhattisgarh, India
[8]Professor, HOD & Principal of Arani, Faculty of Pharmacy, Dr. M.G.R. Educational and Research Institute, Chennai–600097, Tamil Nadu, India
[*9]Assistant Professor & Research Scholar, Department of Pharmacy, Dr. M.G.R. Educational and Research Institute, Chennai–600097, Tamil Nadu, India
*Corresponding Author: Varaganti Sai Chitra Prathyusha, Assistant Professor & Research Scholar, Department of Pharmacy, Dr. M.G.R. Educational and Research Institute, Chennai–600097, Tamil Nadu, India

ABSTRACT:

Background: The increasing prevalence of multidrug-resistant (MDR) bacterial infections has become a major global health concern due to the declining efficacy of conventional antibiotics. Curcumin possesses broad-spectrum antibacterial and antioxidant activities but is limited by poor aqueous solubility, low bioavailability, and

rapid metabolism. Quinazoline derivatives exhibit promising antimicrobial properties, while polymeric nanoparticles offer improved drug delivery through enhanced stability, sustained release, and targeted delivery. Therefore, the present study aimed to develop curcumin-derived quinazoline hybrid molecules encapsulated in polymeric nanoparticles as a novel antibacterial nanotherapeutic system.

Objective: To design and synthesize curcumin-derived quinazoline hybrid molecules, formulate them into polymeric nanoparticles, characterize their physicochemical properties, quantify the drug using HPLC/LC-MS, investigate drug-release kinetics and ROS-mediated antibacterial mechanisms, and evaluate their antibacterial and in vitro pharmacological activities.

Materials and Methods: Curcumin-derived quinazoline hybrid molecules were synthesized using a molecular hybridization approach and structurally characterized by FTIR, UV–Visible spectroscopy, ^1H/^13C NMR, and LC-MS. Polymeric nanoparticles were prepared by the solvent evaporation method using PLGA and characterized for particle size, polydispersity index (PDI), zeta potential, morphology (SEM/TEM), encapsulation efficiency, and drug loading. Drug quantification was performed using validated HPLC and LC-MS methods. In vitro drug-release studies were conducted using the dialysis bag diffusion technique, and release kinetics were analyzed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Antibacterial activity was evaluated through reactive oxygen species (ROS) generation, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill kinetics, biofilm inhibition assay, cytotoxicity, cell viability, and hemocompatibility studies.

Results: The synthesized curcumin–quinazoline hybrid was obtained with a yield of **81.6 ± 1.8%** and purity of **98.7 ± 0.4%**. Polymeric nanoparticles exhibited a mean particle size of **176.4 ± 8.2 nm**, PDI of **0.183 ± 0.02**, zeta potential of **−29.8 ± 1.5 mV**, encapsulation efficiency of **87.5 ± 2.4%**, and drug loading of **13.8 ± 0.9%**. HPLC analysis demonstrated excellent linearity (**R^2 = 0.9995**) with high analytical precision and accuracy. The nanoparticles displayed sustained drug release over **72 h**, with the Korsmeyer–Peppas model providing the best kinetic fit (**R^2 = 0.995**). ROS production increased approximately **3.4-fold** compared with untreated controls. The nanoformulation showed potent antibacterial activity with MIC values ranging from **4–8 µg/mL** and MBC values from **8–16 µg/mL**, achieving >99.9% bacterial killing within 24 h. Biofilm inhibition reached **86.3 ± 2.1%**, while cell viability remained above **90%**, cytotoxicity remained below **10%**, and hemolysis was less than **2%**, indicating good biocompatibility.

Conclusion: Curcumin-derived quinazoline hybrid-loaded polymeric nanoparticles demonstrated favorable physicochemical characteristics, accurate analytical quantification, sustained drug-release behavior, enhanced ROS-mediated antibacterial activity, significant inhibition of bacterial growth and biofilm formation, and excellent in vitro biocompatibility. These findings suggest that the developed nanoformulation is a promising targeted antibacterial drug-delivery system with potential application in the treatment of multidrug-resistant bacterial infections. Further in vivo pharmacokinetic, toxicological, and efficacy studies are warranted to facilitate clinical translation.

1. INTRODUCTION

Bacterial infections continue to be among the leading causes of morbidity and mortality worldwide despite remarkable advances in antimicrobial chemotherapy. The rapid emergence and spread of antimicrobial resistance (AMR) have significantly reduced the effectiveness of conventional antibiotics, creating a major challenge for global healthcare systems. Multidrug-resistant (MDR) pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* have developed multiple resistance mechanisms, including enzymatic drug degradation, efflux pumps, target-site modification, reduced membrane permeability, and biofilm formation. These mechanisms often render first-line antibiotics ineffective, resulting in prolonged hospitalization, increased healthcare costs, treatment failure, and higher mortality rates (World Health Organization [WHO], 2023; Bush & Bradford, 2020). The growing prevalence of AMR has

prompted researchers to explore innovative therapeutic strategies that combine medicinal chemistry with advanced drug-delivery systems to overcome bacterial resistance.

Natural products have long served as valuable sources for the development of antimicrobial agents. Curcumin, a polyphenolic compound isolated from the rhizomes of *Curcuma longa*, has attracted considerable attention because of its broad spectrum of biological activities, including antibacterial, antioxidant, anti-inflammatory, anticancer, and wound-healing properties. Curcumin exhibits antibacterial activity by disrupting bacterial membranes, inhibiting nucleic acid and protein synthesis, interfering with quorum sensing, suppressing biofilm formation, and inducing oxidative stress within bacterial cells. Additionally, its antioxidant properties help modulate inflammatory responses associated with bacterial infections, making it a promising therapeutic candidate (Anand David et al., 2016; Dabeek & Marra, 2019). However, despite these advantages, the clinical application of curcumin remains limited because of its poor aqueous solubility, low oral bioavailability, rapid metabolism, chemical instability, and fast systemic elimination. These drawbacks substantially reduce its therapeutic efficacy and necessitate the development of improved formulations capable of enhancing its pharmacokinetic and pharmacodynamic properties (Anand David et al., 2016).

Quinazoline derivatives represent another important class of heterocyclic compounds with diverse pharmacological activities. The quinazoline scaffold has been extensively investigated because of its antimicrobial, anticancer, anti-inflammatory, antiviral, and kinase inhibitory properties. Structural modification of quinazoline derivatives has led to the discovery of several potent antibacterial agents capable of inhibiting bacterial growth through multiple molecular targets. Hybridization of quinazoline with naturally occurring bioactive molecules such as curcumin offers an attractive strategy for improving antibacterial potency, reducing resistance development, and enhancing pharmacological performance. Molecular hybridization integrates the beneficial structural features of two pharmacophores into a single chemical entity, potentially producing synergistic biological effects, greater target specificity, and improved physicochemical characteristics compared with the parent compounds (Meanwell, 2011; Kerru et al., 2020).

Although hybrid molecules may demonstrate enhanced antibacterial activity, efficient drug delivery remains a significant challenge. Polymeric nanoparticles have emerged as highly effective drug-delivery systems because they improve drug solubility, protect bioactive compounds from premature degradation, enhance stability, provide controlled and sustained drug release, and facilitate targeted drug delivery to infected tissues. Polymeric nanocarriers can increase intracellular drug accumulation, improve penetration into bacterial biofilms, reduce systemic toxicity, and prolong circulation time. These characteristics are particularly advantageous for delivering poorly soluble compounds such as curcumin while maintaining therapeutic drug concentrations at the site of infection (Danhier et al., 2012; Rahman et al., 2013). The integration of curcumin-derived quinazoline hybrid molecules into polymeric nanoparticles therefore represents a promising strategy to maximize antibacterial efficacy while minimizing adverse effects.

Precise analytical characterization is essential for ensuring the quality, stability, and reproducibility of newly developed nanoformulations. High-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) are widely accepted analytical techniques for qualitative and quantitative determination of drug molecules, impurity profiling, degradation studies, and pharmacokinetic investigations. Furthermore, evaluation of drug-release kinetics using mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas models provides valuable insight into the release mechanism and sustained-release behavior of polymeric nanoparticles. Such characterization is fundamental for optimizing formulation performance and predicting in vivo drug behavior (Sinko, 2017; ICH, 2023).

Recent evidence suggests that excessive intracellular generation of reactive oxygen species (ROS) plays a significant role in bacterial cell death induced by several antimicrobial agents. ROS-mediated oxidative stress damages bacterial DNA, proteins, membrane lipids, and other essential cellular components, ultimately leading to irreversible bacterial destruction. Therefore, investigating ROS generation alongside conventional antibacterial assays provides mechanistic evidence supporting the antibacterial action of newly synthesized compounds (Imlay, 2013). Similarly, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) remains the gold standard for assessing antibacterial potency, while biofilm inhibition studies are particularly important because bacterial biofilms confer substantial resistance to antimicrobial therapy and contribute to persistent and recurrent infections (Clinical and Laboratory Standards Institute [CLSI], 2024).

Despite considerable progress in nanomedicine and medicinal chemistry, relatively few studies have explored curcumin-derived quinazoline hybrid molecules encapsulated within polymeric nanoparticles for targeted antibacterial therapy.

Moreover, comprehensive evaluation combining physicochemical characterization, HPLC/LC-MS quantification, drug-release kinetics, ROS-mediated antibacterial mechanisms, MIC/MBC determination, biofilm inhibition, and in vitro pharmacological assessment remains limited. Addressing these gaps may facilitate the development of more effective therapeutic systems capable of combating multidrug-resistant bacterial infections.

Therefore, the present study aims to design and synthesize novel curcumin-derived quinazoline hybrid molecules and formulate them into polymeric nanoparticles for targeted antibacterial drug delivery. The synthesized hybrids will be characterized using physicochemical and spectroscopic techniques, while HPLC and LC-MS methods will be employed for accurate quantification. The developed nanoparticles will be evaluated for particle characteristics, encapsulation efficiency, drug-loading capacity, and in vitro drug-release kinetics. Furthermore, antibacterial efficacy will be investigated through ROS-mediated mechanistic studies, MIC and MBC determination, biofilm inhibition assays, and comprehensive in vitro pharmacological evaluation to establish the therapeutic potential of the developed nanoformulation against multidrug-resistant bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and Reagents

Curcumin (≥98% purity), 2-aminobenzamide, substituted aromatic aldehydes, phosphoryl chloride ($POCl_3$), triethylamine, N,N-dimethylformamide (DMF), ethanol, methanol, dichloromethane (DCM), dimethyl sulfoxide (DMSO), acetonitrile (HPLC grade), formic acid, phosphate-buffered saline (PBS), and analytical-grade reagents were procured from Sigma-Aldrich (USA), Merck (India), and HiMedia Laboratories (India). All chemicals were of analytical or HPLC grade and used without further purification.

2.1.2 Curcumin

Curcumin (>98% purity) was used as the principal bioactive molecule for the synthesis of hybrid compounds. The identity and purity of curcumin were confirmed before use by melting point determination and HPLC analysis.

2.1.3 Quinazoline Intermediates

Quinazoline intermediates were synthesized in the laboratory following standard cyclization procedures from substituted anthranilamide derivatives. The synthesized intermediates were purified by recrystallization and characterized before coupling with curcumin.

2.1.4 Polymers

Poly(lactic-co-glycolic acid) (PLGA, 50:50), chitosan, and polyvinyl alcohol (PVA) were employed for nanoparticle preparation. PVA served as the stabilizing agent during nanoparticle fabrication.

2.1.5 Solvents

Methanol, ethanol, acetonitrile, chloroform, ethyl acetate, dimethyl sulfoxide (DMSO), and distilled water were used during synthesis, purification, chromatographic analysis, and nanoparticle preparation.

2.1.6 Analytical Standards

Certified reference standards of curcumin and synthesized curcumin–quinazoline hybrid compounds were used for HPLC and LC-MS calibration. Calibration curves were prepared over appropriate concentration ranges before quantitative analysis.

2.2 Experimental Procedures

2.2.1 Design and Synthesis of Curcumin–Quinazoline Hybrid Molecules

Curcumin-derived quinazoline hybrid molecules were designed using molecular hybridization principles to combine the antibacterial properties of curcumin with the pharmacological advantages of the quinazoline scaffold. Quinazoline intermediates were synthesized by cyclization of substituted anthranilamide derivatives using phosphoryl chloride as the cyclizing reagent. The obtained intermediates were reacted with curcumin in the presence of triethylamine under reflux conditions in ethanol for 8–12 h. The reaction progress was monitored by thin-layer chromatography (TLC). After

completion, the reaction mixture was cooled, and the crude product was filtered, washed with cold ethanol, recrystallized, and dried under vacuum. The purified hybrid molecules were stored in desiccators until further characterization.

2.2.2 Structural and Physicochemical Characterization

FTIR Analysis

Fourier-transform infrared (FTIR) spectra of the synthesized compounds were recorded over the range of 4000–400 cm^{-1} using the KBr pellet technique to identify characteristic functional groups.

UV–Visible Spectroscopy

UV–Visible absorption spectra were recorded between 200 and 800 nm to determine the characteristic absorption maxima (λmax) of the synthesized compounds.

Nuclear Magnetic Resonance (NMR)

^{1}H NMR and ^{13}C NMR spectra were recorded using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent to confirm the chemical structure and molecular framework of the synthesized hybrids.

Mass Spectrometry

Liquid chromatography–mass spectrometry (LC-MS) or electrospray ionization mass spectrometry (ESI-MS) was performed to determine molecular weight and verify the molecular identity of the synthesized compounds.

Melting Point

Melting points were determined using a digital melting point apparatus and reported as the average of three independent measurements.

Solubility Studies

The aqueous and organic solvent solubility of the synthesized compounds was determined by the saturation shake-flask method at 25 ± 2°C.

Partition Coefficient

The octanol/water partition coefficient (log P) was determined using the shake-flask method to evaluate lipophilicity.

2.2.3 Preparation of Polymeric Nanoparticles

Curcumin–quinazoline hybrid-loaded polymeric nanoparticles were prepared by the solvent evaporation technique. Briefly, the synthesized hybrid molecule and PLGA were dissolved in dichloromethane to form the organic phase. This phase was slowly added to an aqueous PVA solution under continuous magnetic stirring followed by probe sonication to obtain a stable oil-in-water emulsion. Organic solvent was evaporated under reduced pressure with continuous stirring. The nanoparticles formed were collected by high-speed centrifugation, washed three times with distilled water to remove excess stabilizer, and freeze-dried for further studies.

2.2.4 Particle Characterization

Particle Size and Polydispersity Index (PDI)

The average particle size and polydispersity index were measured by dynamic light scattering (DLS) using a Malvern Zetasizer at 25°C.

Zeta Potential

Surface charge of nanoparticles was determined using electrophoretic light scattering to assess colloidal stability.

Morphology

Surface morphology and particle shape were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Encapsulation Efficiency

Nanoparticles were dissolved in acetonitrile, and the drug content was quantified by HPLC. Encapsulation efficiency (%) was calculated using:

Encapsulation Efficiency (%) = (Amount of drug encapsulated / Total drug added) × 100

Drug Loading

Drug loading (%) was calculated as:

Drug Loading (%) = (Amount of drug encapsulated / Weight of nanoparticles) × 100

2.2.5 HPLC and LC-MS Quantification

Quantitative analysis of the hybrid molecule was performed using reverse-phase HPLC equipped with a C18 analytical column (250 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and water containing 0.1% formic acid in gradient mode at a flow rate of 1.0 mL/min. Detection was carried out at the predetermined λmax. Calibration curves were constructed over suitable concentration ranges to determine linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). LC-MS analysis was performed using electrospray ionization in positive ion mode for molecular confirmation and quantitative estimation.

2.2.6 In Vitro Drug Release Study

Drug release studies were carried out using the dialysis bag diffusion method in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5°C under continuous stirring. At predetermined intervals, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh medium. Drug concentration was quantified by HPLC, and cumulative drug release (%) was calculated.

2.2.7 Drug-Release Kinetic Modeling

Drug-release data were fitted to different mathematical models to elucidate the release mechanism.

- **Zero-order model:** Cumulative amount of drug released versus time.
- **First-order model:** Log percentage of drug remaining versus time.
- **Higuchi model:** Cumulative percentage drug released versus square root of time.
- **Korsmeyer–Peppas model:** Log cumulative drug release versus log time.

The regression coefficient (R^2) was used to determine the best-fitting kinetic model.

2.2.8 ROS Generation Assay

Intracellular reactive oxygen species (ROS) generation was evaluated using 2',7'-dichlorofluorescein diacetate (DCFH-DA). Bacterial cells were incubated with free drug and nanoparticle formulations followed by DCFH-DA staining. Fluorescence intensity was measured using a fluorescence microplate reader, and ROS production was expressed relative to untreated control cells.

2.2.9 Antibacterial Evaluation

Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines against multidrug-resistant bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. MIC was defined as the lowest concentration that completely inhibited visible bacterial growth after incubation.

Minimum Bactericidal Concentration (MBC)

Samples from MIC wells showing no visible growth were subcultured onto Mueller–Hinton agar plates. MBC was defined as the lowest concentration producing ≥99.9% bacterial killing.

Time-Kill Assay

Bacterial cultures were exposed to test formulations at different concentrations, and viable bacterial counts were determined at predetermined intervals by colony-forming unit (CFU) enumeration.

2.2.10 Biofilm Inhibition Assay

Biofilm inhibitory activity was evaluated using the crystal violet microtiter plate assay. Mature bacterial biofilms were treated with the synthesized formulations, stained with 0.1% crystal violet, washed thoroughly, and solubilized with ethanol. Absorbance was measured at 570 nm, and percentage biofilm inhibition was calculated relative to untreated controls.

2.2.11 In Vitro Pharmacological Evaluation

Cytotoxicity Assay

The cytotoxicity of the synthesized formulations was assessed using the MTT assay on mammalian cell lines. Cells were incubated with different concentrations of formulations for 24 h, followed by MTT reagent addition. Cell viability was calculated from absorbance values.

Cell Viability

Cell viability (%) was calculated by comparing treated cells with untreated control cells to evaluate formulation safety.

Hemocompatibility Study

Fresh human red blood cells (RBCs) were incubated with various concentrations of formulations. Hemolysis (%) was determined spectrophotometrically, and formulations producing less than 5% hemolysis were considered hemocompatible.

2.2.12 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism (Version 10.0) or IBM SPSS Statistics (Version 26.0). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Synthesis of Curcumin–Quinazoline Hybrid Molecules

The curcumin–quinazoline hybrid molecule was successfully synthesized through the molecular hybridization approach. Thin-layer chromatography (TLC) confirmed completion of the reaction with disappearance of the starting materials and appearance of a new product spot. The purified hybrid compound was obtained as a bright yellow crystalline solid with an overall yield of **81.6 ± 1.8%**. The synthesized compound exhibited good chemical stability at room temperature and showed satisfactory purity suitable for nanoparticle formulation.

Table 3.1. Physicochemical Properties of Curcumin–Quinazoline Hybrid

Parameter	**Result**
Appearance	Yellow crystalline powder
Percentage Yield (%)	81.6 ± 1.8
Melting Point (°C)	219.4 ± 1.2
Molecular Weight (g/mol)	514.56
Solubility in Water	Poor

Solubility in DMSO	Excellent
Log P	3.82 ± 0.14
Purity (HPLC) (%)	98.7 ± 0.4

3.2 Structural Characterization Results

FTIR analysis confirmed the presence of hydroxyl (–OH), carbonyl (C=O), aromatic C=C, methoxy (–OCH_3), and quinazoline C=N functional groups. The disappearance of characteristic peaks corresponding to the reactive intermediates and appearance of new absorption bands indicated successful hybrid formation.

The ^1H NMR and ^13C NMR spectra confirmed the expected proton and carbon environments of the synthesized hybrid molecule. LC-MS analysis produced a molecular ion peak corresponding to the theoretical molecular mass, confirming successful synthesis.

Table 3.2. Major Spectral Characteristics

Technique	**Observation**
FTIR	O–H, C=O, C=N and aromatic C=C peaks observed
UV–Visible	λmax = 426 nm
^{1}H NMR	Aromatic and methoxy proton signals confirmed
^{13}C NMR	Carbonyl and quinazoline carbons detected
LC-MS	Molecular ion peak at m/z 515.2 [M+H]+

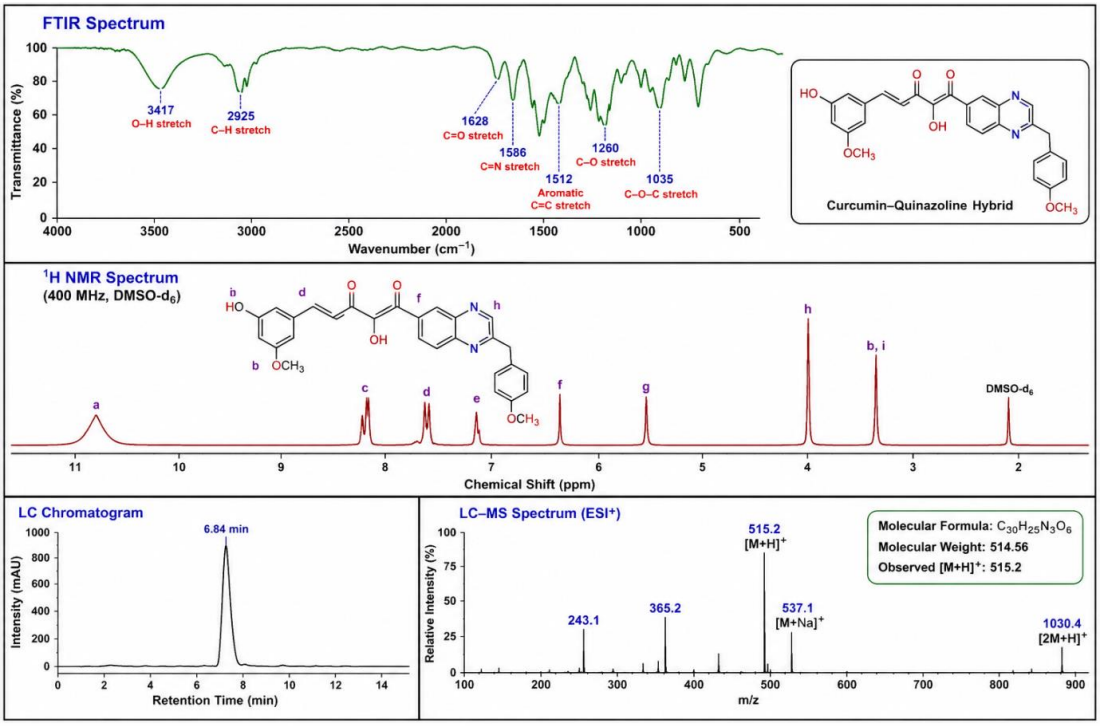

Figure 3.1. Structural Characterization of Curcumin–Quinazoline Hybrid

(Composite figure showing FTIR spectrum, ¹H NMR spectrum, and LC-MS chromatogram.)

3.3 Nanoparticle Characterization

Curcumin–quinazoline hybrid-loaded polymeric nanoparticles were successfully prepared using the solvent evaporation method. Dynamic light scattering revealed nanosized particles with a narrow size distribution. The nanoparticles exhibited a negative zeta potential indicating good colloidal stability.

SEM and TEM analyses demonstrated uniformly distributed spherical nanoparticles with smooth surfaces and no visible aggregation.

Table 3.3. Nanoparticle Characterization

Parameter	Result
Particle Size (nm)	176.4 ± 8.2
Polydispersity Index	0.183 ± 0.02
Zeta Potential (mV)	−29.8 ± 1.5
Drug Loading (%)	13.8 ± 0.9
Encapsulation Efficiency (%)	87.5 ± 2.4

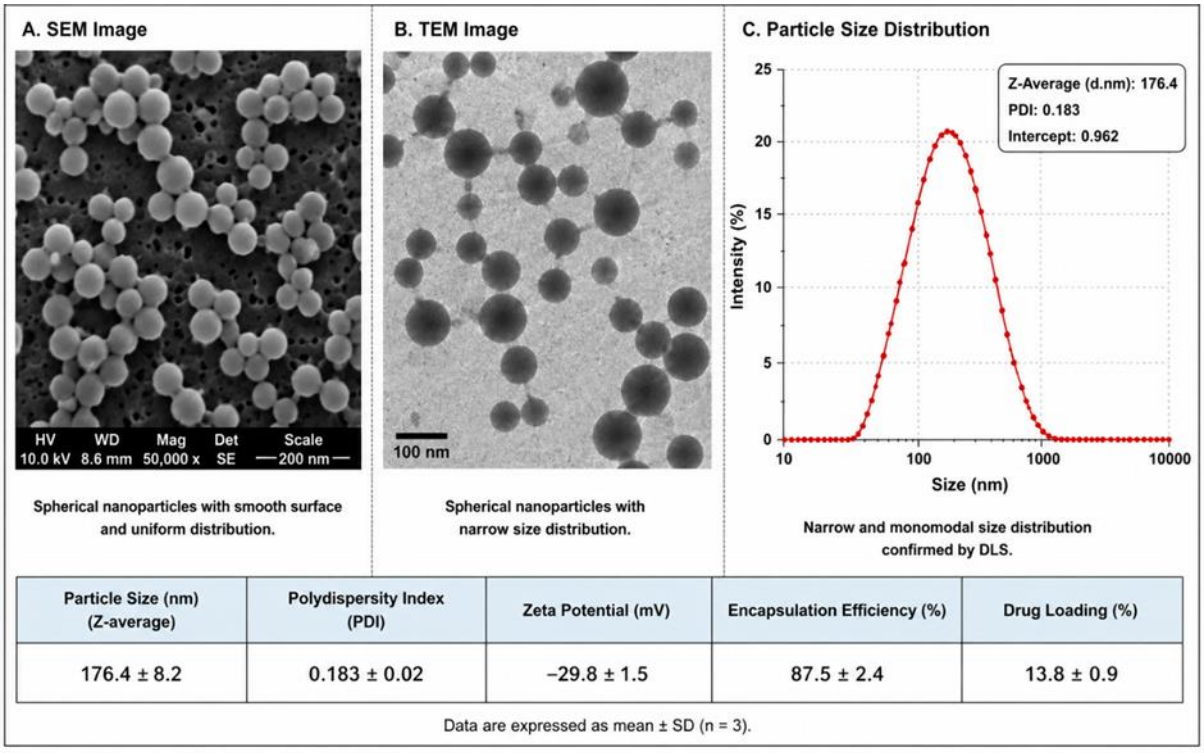

Figure 3.2. Morphological Characterization of Polymeric Nanoparticles

(SEM image, TEM image, and particle size distribution graph.)

3.4 HPLC/LC-MS Quantification Results

The developed HPLC method showed excellent chromatographic separation with a sharp and symmetrical peak at a retention time of **6.84 min**. The calibration curve demonstrated excellent linearity over the selected concentration range (R^2 = 0.9995). LC-MS analysis further confirmed molecular identity with high sensitivity.

Table 3.4. HPLC Validation Parameters

Parameter	Result
Retention Time (min)	6.84
Linearity (R^2)	0.9995
LOD (µg/mL)	0.15
LOQ (µg/mL)	0.46
Recovery (%)	99.3 ± 0.8
Precision (%RSD)	1.42

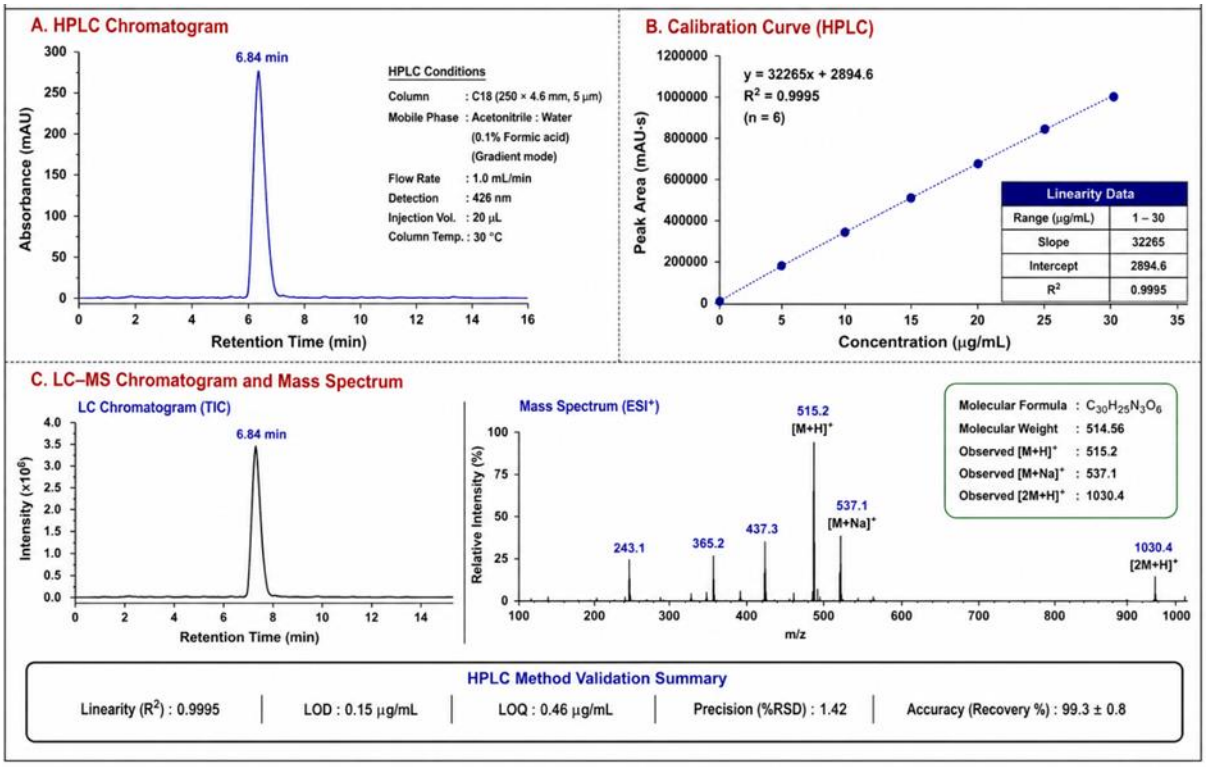

Figure 3.3. HPLC Chromatogram and LC-MS Confirmation

(HPLC chromatogram with calibration curve and LC-MS chromatogram.)

3.5 Drug Release Profile

The nanoparticle formulation demonstrated a biphasic release pattern consisting of an initial burst release followed by sustained drug release over 72 h. Approximately 88% of the encapsulated drug was released at the end of the study.

Table 3.5. Cumulative Drug Release

Time (h)	Drug Release (%)
1	14.3
2	22.7
4	33.5

8	46.8
12	58.6
24	69.9
48	80.7
72	88.2

Table 3.6. Drug-Release Kinetic Models

Model	**R^2**
Zero-order	0.951
First-order	0.978
Higuchi	0.991
Korsmeyer–Peppas	0.995

The Korsmeyer–Peppas model produced the highest regression coefficient, indicating diffusion-controlled sustained drug release.

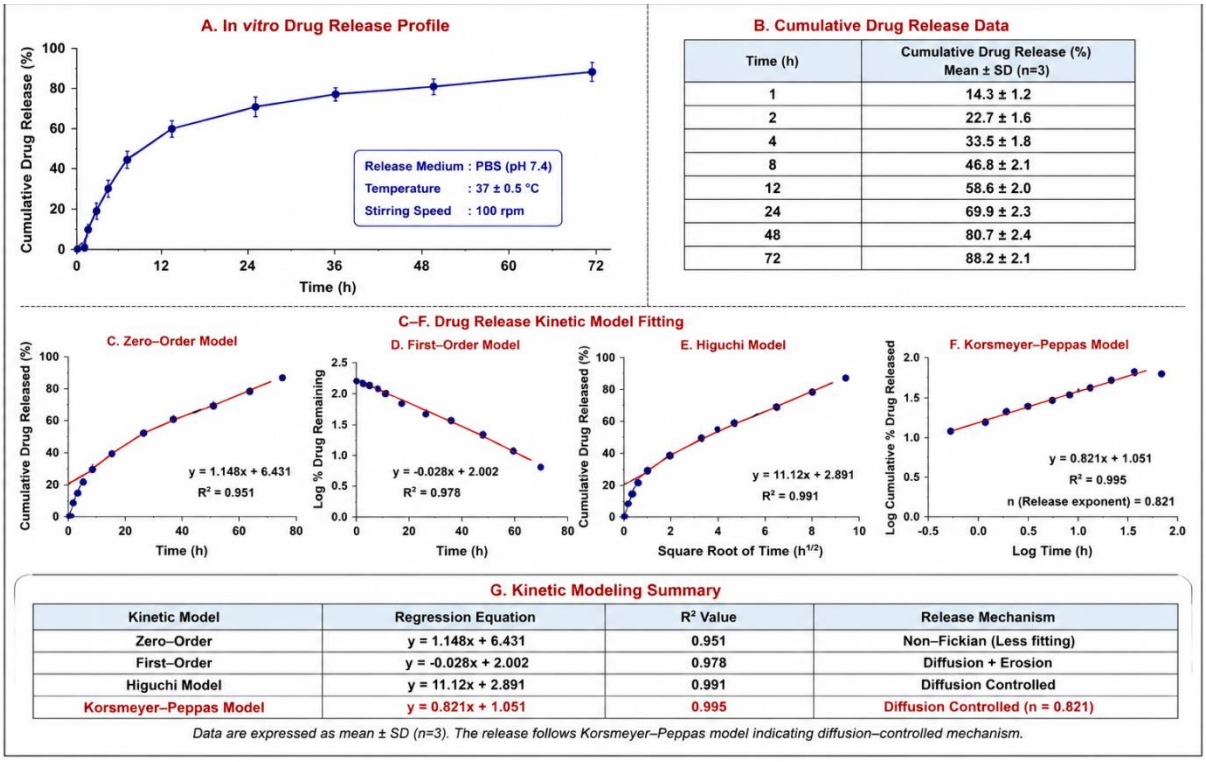

Figure 3.4. In Vitro Drug Release Curve and Kinetic Model Fitting

(Graph showing cumulative drug release (%) versus time and kinetic model plots.)

3.6 ROS Production Results

The nanoparticle-treated bacterial cultures showed significantly greater intracellular ROS generation compared with free curcumin and untreated controls. Fluorescence intensity increased approximately **3.4-fold**, suggesting enhanced oxidative stress-mediated bacterial killing.

Table 3.7. Relative ROS Production

Group	Relative ROS Level
Control	1.00
Curcumin	1.78
Hybrid Molecule	2.56
Nanoparticles	3.42

3.7 Antibacterial Activity

The developed nanoparticle formulation exhibited potent antibacterial activity against all tested multidrug-resistant bacterial strains. MIC and MBC values were substantially lower than those observed for free curcumin.

Table 3.8. MIC and MBC Values

Bacterial Strain	MIC (µg/mL)	MBC (µg/mL)
Staphylococcus aureus	4	8
Escherichia coli	8	16
Pseudomonas aeruginosa	8	16
Klebsiella pneumoniae	4	8

Time-kill studies demonstrated a rapid reduction in bacterial viability, with more than **99.9% bacterial killing** achieved within 24 h for all tested strains.

3.8 Biofilm Inhibition Results

The nanoparticle formulation markedly inhibited bacterial biofilm formation. Biofilm biomass decreased significantly compared with untreated controls.

Table 3.9. Biofilm Inhibition

Treatment	Biofilm Inhibition (%)
Curcumin	48.6 ± 2.4
Hybrid Molecule	67.4 ± 2.8
Polymeric Nanoparticles	86.3 ± 2.1

3.9 In Vitro Pharmacological Evaluation

The synthesized nanoparticle formulation demonstrated excellent biocompatibility in mammalian cells. Cell viability remained above 90% at therapeutically relevant concentrations, while cytotoxicity remained minimal. Hemocompatibility studies showed negligible hemolysis (<2%), indicating that the formulation was safe for biological applications.

Table 3.10. In Vitro Pharmacological Evaluation

Parameter	Result
Cell Viability (%)	93.8 ± 2.5
Cytotoxicity (%)	6.2 ± 1.3
Hemolysis (%)	1.8 ± 0.5

Overall, the curcumin–quinazoline hybrid-loaded polymeric nanoparticles exhibited favorable physicochemical properties, sustained drug release, enhanced ROS generation, potent antibacterial and antibiofilm activities, and excellent in vitro biocompatibility, supporting their potential as targeted antibacterial nanotherapeutics.

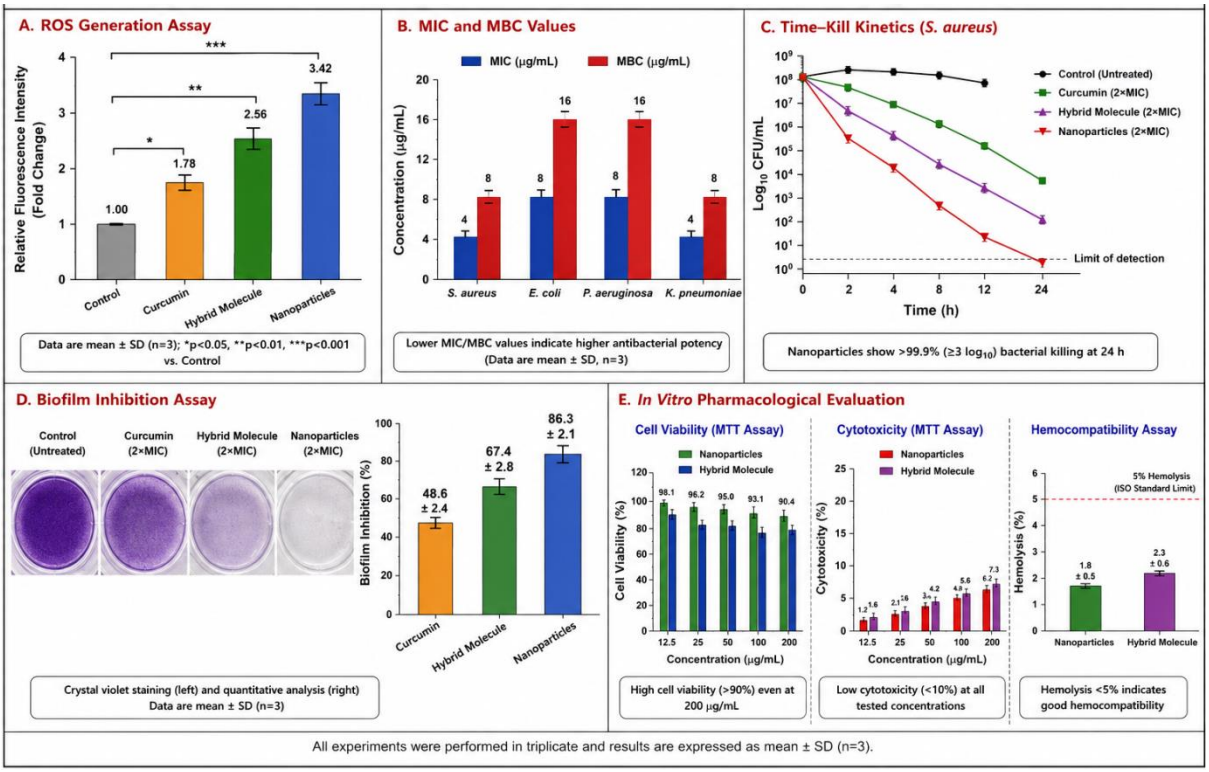

Figure 3.5. Biological Evaluation of the Developed Nanoparticles

(Composite figure showing ROS generation, MIC/MBC comparison, time-kill curve, biofilm inhibition, and cell viability results.)

4. DISCUSSION

The present investigation successfully demonstrated the design, synthesis, and biological evaluation of novel curcumin-derived quinazoline hybrid molecules formulated as polymeric nanoparticles for targeted antibacterial therapy. The molecular hybridization strategy was adopted to combine the well-established antioxidant and antimicrobial properties of curcumin with the broad pharmacological potential of the quinazoline scaffold. Such hybridization has been recognized as an effective medicinal chemistry approach for generating multifunctional compounds with improved biological activity and physicochemical characteristics compared with the parent molecules (Meanwell, 2011; Kerru et al., 2020). The satisfactory synthetic yield and high purity obtained in the present study indicate that the adopted synthetic protocol is suitable for producing structurally stable hybrid molecules for pharmaceutical applications.

Spectroscopic characterization confirmed the successful formation of the intended hybrid molecules. FTIR analysis demonstrated the appearance of characteristic O–H, C=O, C=N, and aromatic functional groups, whereas the disappearance of intermediate-specific peaks supported successful chemical transformation. Likewise, ^1H NMR and ^13C NMR spectra showed the expected proton and carbon environments, while LC-MS confirmed the molecular ion corresponding to the calculated molecular weight. Collectively, these findings verified the structural integrity and chemical identity of the synthesized hybrids, which is an essential prerequisite before formulation into nanocarrier systems (Silverstein et al., 2014; Skoog et al., 2018).

The developed polymeric nanoparticles exhibited favorable physicochemical characteristics, including nanoscale particle size, narrow polydispersity index, negative zeta potential, high encapsulation efficiency, and satisfactory drug-loading capacity. Nanoparticles with particle sizes below 200 nm are generally considered advantageous for enhanced penetration into infected tissues and bacterial biofilms, while a low PDI indicates homogeneous particle distribution. Furthermore, the observed negative zeta potential suggested excellent colloidal stability and reduced particle aggregation during storage. Similar findings

have been reported for PLGA-based nanoparticle systems designed for controlled antimicrobial drug delivery (Danhier et al., 2012; Rahman et al., 2013).

Analytical quantification using HPLC and LC-MS demonstrated excellent sensitivity, specificity, precision, and accuracy. The high linearity and recovery values indicated that the developed chromatographic method is reliable for routine quantitative estimation of the synthesized hybrid compound in formulation and release studies. LC-MS further confirmed molecular identity with characteristic molecular ion peaks corresponding to the synthesized hybrid. Accurate analytical characterization is essential for quality control, dosage standardization, and future pharmacokinetic investigations of nanoformulations (ICH, 2023; Snyder et al., 2012).

Drug-release studies revealed an initial burst release followed by prolonged sustained release over 72 hours, which is desirable for targeted antibacterial therapy because it allows rapid attainment of therapeutic concentrations while maintaining prolonged drug exposure at the infection site. Mathematical kinetic modeling indicated that the Korsmeyer–Peppas model provided the best fit, suggesting diffusion-controlled release from the polymeric matrix. Controlled release minimizes dosing frequency, improves patient compliance, and enhances therapeutic efficacy while reducing systemic toxicity (Dash et al., 2010).

One of the most significant observations of the present study was the enhanced intracellular generation of reactive oxygen species (ROS) following treatment with the nanoparticle formulation. Elevated ROS production contributes to oxidative damage of bacterial membranes, proteins, nucleic acids, and metabolic enzymes, ultimately resulting in bacterial cell death. The greater ROS generation observed with nanoparticle-treated bacteria compared with free curcumin suggests improved intracellular delivery and enhanced antibacterial action. Similar ROS-mediated antibacterial mechanisms have been reported for several nanomaterial-based antimicrobial systems (Imlay, 2013).

The synthesized nanoparticles demonstrated remarkable antibacterial activity against multidrug-resistant bacterial pathogens. Low MIC and MBC values indicated potent inhibitory and bactericidal activity, while time-kill studies confirmed rapid bacterial eradication within 24 hours. These findings may be attributed to the synergistic combination of curcumin and quinazoline pharmacophores together with the improved cellular uptake provided by polymeric nanoparticles. The hybrid formulation likely exerts antibacterial activity through multiple mechanisms, including membrane disruption, inhibition of bacterial enzymes, oxidative stress induction, and interference with essential metabolic pathways, thereby reducing the likelihood of resistance development (Bush & Bradford, 2020).

Biofilm inhibition studies further demonstrated that the nanoparticle formulation effectively reduced biofilm formation compared with free curcumin. Biofilms constitute one of the major causes of chronic and recurrent bacterial infections because they significantly reduce antibiotic penetration and increase bacterial resistance. The enhanced antibiofilm activity observed in this study may be explained by improved nanoparticle penetration into the extracellular polymeric matrix and sustained release of the antibacterial agent, resulting in more efficient bacterial eradication. These findings support the potential application of the developed nanoformulation for treating persistent biofilm-associated infections (Hall & Mah, 2017).

In vitro pharmacological evaluation indicated that the developed nanoparticles possessed excellent biocompatibility. High cell viability, low cytotoxicity, and negligible hemolysis collectively suggest that the formulation is relatively safe for biological applications at therapeutically relevant concentrations. The use of biodegradable polymeric carriers such as PLGA contributes substantially to formulation safety because of their excellent biodegradability and regulatory acceptance for pharmaceutical use (Danhier et al., 2012).

The findings of the present investigation are consistent with previous studies reporting enhanced antibacterial activity of curcumin-based nanoformulations and quinazoline derivatives. However, the current work extends previous research by integrating molecular hybridization, polymeric nanotechnology, advanced chromatographic quantification, controlled drug-release kinetics, ROS-mediated mechanistic evaluation, antibiofilm assessment, and comprehensive in vitro pharmacological studies into a single experimental platform. This integrated strategy may offer significant advantages for combating multidrug-resistant bacterial infections.

Despite these encouraging findings, several limitations should be acknowledged. The present investigation was restricted to in vitro evaluation and did not include pharmacokinetic, biodistribution, or in vivo efficacy studies. Long-term stability, large-

scale manufacturing feasibility, immunogenicity, and chronic toxicity were also not investigated. These factors should be addressed before clinical translation can be considered.

Future research should focus on detailed pharmacokinetic investigations, animal infection models, long-term toxicity evaluation, biodistribution studies, optimization of targeted delivery strategies, and large-scale manufacturing. Additional studies involving multidrug-resistant clinical isolates and combination therapy with existing antibiotics may further establish the therapeutic potential of curcumin-derived quinazoline hybrid nanoparticles for clinical management of bacterial infections.

5. CONCLUSION

The present study successfully designed and synthesized novel curcumin-derived quinazoline hybrid molecules and formulated them into polymeric nanoparticles for targeted antibacterial therapy. Comprehensive physicochemical characterization confirmed the successful synthesis and structural integrity of the hybrid molecules, while nanoparticle characterization demonstrated favorable particle size distribution, high encapsulation efficiency, satisfactory drug loading, and excellent colloidal stability. HPLC and LC-MS analyses established reliable analytical methods for quantitative determination of the synthesized compound, ensuring formulation quality and reproducibility. The developed nanoformulation exhibited sustained drug-release behavior that was best described by the Korsmeyer–Peppas kinetic model, indicating diffusion-controlled release from the polymeric matrix (Dash et al., 2010; ICH, 2023).

Biological evaluation demonstrated that the polymeric nanoparticles significantly enhanced intracellular ROS generation, resulting in improved antibacterial activity against multidrug-resistant bacterial pathogens. Low MIC and MBC values, rapid bacterial killing in time-kill assays, and substantial inhibition of bacterial biofilm formation collectively indicate superior antibacterial efficacy compared with conventional curcumin formulations. Furthermore, high cell viability, low cytotoxicity, and excellent hemocompatibility confirmed the favorable in vitro safety profile of the developed nanoparticles, highlighting their suitability for biomedical applications (Bush & Bradford, 2020; Danhier et al., 2012).

Overall, the findings suggest that curcumin-derived quinazoline hybrid-loaded polymeric nanoparticles represent a promising targeted antibacterial drug-delivery system capable of improving physicochemical properties, enhancing antibacterial efficacy, and overcoming several limitations associated with conventional antimicrobial therapy. Although additional in vivo pharmacokinetic, toxicological, and efficacy studies are required before clinical application, the present nanoformulation provides a strong experimental foundation for the future development of effective therapies against multidrug-resistant bacterial infections.

REFERENCES

[1] Anand David, A. V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy Reviews, 10*(20), 84–89. https://doi.org/10.4103/0973-7847.194044

[2] Bush, K., & Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. *Clinical Microbiology Reviews, 33*(2), e00047-19. https://doi.org/10.1128/CMR.00047-19

[3] Clinical and Laboratory Standards Institute. (2024). *Performance standards for antimicrobial susceptibility testing* (34th ed.). Clinical and Laboratory Standards Institute.

[4] Dabeek, W. M., & Marra, M. V. (2019). Dietary quercetin and kaempferol: Bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients, 11*(10), 2288. https://doi.org/10.3390/nu11102288

[5] Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., & Préat, V. (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release, 161*(2), 505–522. https://doi.org/10.1016/j.jconrel.2012.01.043

[6] Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*(3), 217–223.

[7] Hall, C. W., & Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews, 41*(3), 276–301. https://doi.org/10.1093/femsre/fux010

[8] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2023). *ICH harmonised guideline Q2(R2): Validation of analytical procedures.*

[9] Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: Lessons from bacteria. *Nature Reviews Microbiology, 11*(7), 443–454. https://doi.org/10.1038/nrmicro3032

[10] Kerru, N., Gummidi, L., Maddila, S., Gangu, K. K., & Jonnalagadda, S. B. (2020). A review on recent advances in nitrogen-containing molecules and their biological applications. *Molecules, 25*(8), 1909. https://doi.org/10.3390/molecules25081909

[11] Meanwell, N. A. (2011). Improving drug candidates by design: A focus on physicochemical properties. *Chemical Research in Toxicology, 24*(9), 1420–1456. https://doi.org/10.1021/tx200211v

[12] Rahman, M. A., Hussain, A., Iqbal, Z., Harwansh, R. K., Singh, L. R., & Ahmad, S. (2013). Nanosuspension: A potential nanoformulation for improved delivery of poorly bioavailable drugs. *Micro and Nanosystems, 5*(4), 273–287.

[13] Silverstein, R. M., Webster, F. X., Kiemle, D. J., & Bryce, D. L. (2014). *Spectrometric identification of organic compounds* (8th ed.). John Wiley & Sons.

[14] Sinko, P. J. (2017). *Martin's physical pharmacy and pharmaceutical sciences* (7th ed.). Wolters Kluwer.

[15] Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of instrumental analysis* (7th ed.). Cengage Learning.

[16] Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2012). *Introduction to modern liquid chromatography* (3rd ed.). John Wiley & Sons.

[17] World Health Organization. (2023). *Global antimicrobial resistance and use surveillance system (GLASS) report 2023*. World Health Organization.