Formulation and Therapeutic Investigation of Curcumin–Piperine Co-Encapsulated Solid Lipid Nanoparticles to Overcome Multidrug Resistance in Colorectal Cancer

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ABSTRACT

Colorectal cancer remains a major global health challenge, with multidrug resistance (MDR) significantly limiting the efficacy of conventional chemotherapy. The present study aimed to develop and evaluate curcumin–piperine co-encapsulated solid lipid nanoparticles (SLNs) to enhance anticancer efficacy and overcome MDR in colorectal cancer. SLNs were prepared using hot homogenization followed by ultrasonication and optimized based on particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The optimized formulation exhibited a particle size of 154.8 nm, PDI of 0.238, zeta potential of −34.5 mV, and high entrapment efficiency for both curcumin (91.4%) and piperine (89.7%). In vitro release studies showed sustained drug release up to 24 hours, following Korsmeyer–Peppas kinetics. Stability studies confirmed minimal physicochemical variation over three months.

Biological evaluation demonstrated significantly enhanced cytotoxicity, with an IC50 of 2.86 μM, representing a 4.13-fold improvement over free curcumin. The formulation showed increased cellular uptake (2.83-fold), elevated reactive oxygen species generation (3.18-fold), and higher apoptosis induction (68.58%). Notably, a 4.20-fold increase in Rhodamine 123 accumulation confirmed effective inhibition of P-glycoprotein-mediated drug efflux. Additionally, the formulation significantly inhibited cell migration and colony formation. Overall, the study highlights the potential of curcumin–piperine SLNs as a promising nanotherapeutic approach for overcoming MDR in colorectal cancer.

Keywords: Curcumin; Piperine; Solid lipid nanoparticles; Colorectal cancer; Multidrug resistance; P-glycoprotein; Nanocarriers; Drug delivery

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Introduction

Colorectal cancer is one of the most prevalent malignancies worldwide and remains a leading cause of cancer-related morbidity and mortality. Despite significant advancements in diagnostic and therapeutic approaches, the clinical management of colorectal cancer continues to be challenged by tumor recurrence, metastasis, and the development of multidrug

resistance (MDR). Conventional chemotherapeutic agents, although initially effective, often lose their efficacy over time due to intrinsic or acquired resistance mechanisms. Among these, overexpression of ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (P-gp), plays a pivotal role by actively effluxing anticancer drugs out of tumor cells, thereby reducing intracellular drug accumulation and therapeutic response. Consequently, overcoming MDR has emerged as a critical objective in improving colorectal cancer treatment outcomes (Bao *et al.*, 2026; Jafari *et al.*, 2023; Khan *et al.*, 2025; Piotrowska & Orzechowska, 2024; Wang *et al.*, 2025).

Natural phytoconstituents have gained considerable attention as potential anticancer agents due to their multitargeted mechanisms of action and relatively low toxicity profiles (Saroj *et al.*, 2023). Curcumin, a polyphenolic compound derived from *Curcuma longa*, has been extensively studied for its anticancer properties. It exerts its effects by modulating multiple signalling pathways involved in carcinogenesis, including NF-κB, PI3K/Akt, Wnt/β-catenin, and apoptotic cascades (Pan *et al.*, 2024; Shahbaz *et al.*, 2022). Curcumin has demonstrated the ability to inhibit cell proliferation, induce apoptosis, suppress inflammation, and reduce oxidative stress in colorectal cancer models. However, its clinical application is severely limited by poor aqueous solubility, low bioavailability, rapid metabolism, and limited cellular uptake (Aati *et al.*, 2025; Fekri Soufiabadi *et al.*, 2025; Hadi *et al.*, 2025; Muztaba *et al.*, 2026; Wu *et al.*, 2025; Zhao *et al.*, 2025).

Piperine, an alkaloid obtained from *Piper nigrum*, has been recognized as a potent bioenhancer capable of improving the pharmacokinetic profile of various drugs. It enhances drug absorption by inhibiting drug-metabolizing enzymes and efflux transporters such as P-glycoprotein. In addition to its bioenhancing properties, piperine also exhibits intrinsic anticancer activity, including inhibition of cell proliferation and induction of apoptosis. The combination of curcumin and piperine has been shown to produce synergistic effects, where piperine enhances the bioavailability and therapeutic efficacy of curcumin while also contributing to MDR modulation (Aisyah *et al.*, 2024; El-Nahas *et al.*, 2024; Li *et al.*, 2021; Nur Aisyah *et al.*, 2025). Nanotechnology-based drug delivery systems have emerged as promising strategies to address the limitations associated with conventional drug administration. Among these, solid lipid nanoparticles (SLNs) have gained significant interest due to their biocompatibility, ability to encapsulate lipophilic drugs, controlled drug release, and enhanced cellular uptake. SLNs consist of a solid lipid core stabilized by surfactants, which allows for efficient drug incorporation and protection from degradation. Additionally, SLNs can improve drug permeability and facilitate passive targeting to tumor tissues through enhanced retention and uptake (Khan *et al.*, 2015; Kumar *et al.*, 2025; Kumar *et al.*, 2022; Panda & Mohapatra, 2024; Shehata *et al.*, 2022).

Co-encapsulation of multiple therapeutic agents within a single nanocarrier offers additional advantages, including synchronized drug delivery, improved pharmacokinetic profiles, and potential synergistic therapeutic effects. In the context of MDR, such systems can simultaneously deliver an anticancer agent and a resistance-modulating compound, thereby enhancing intracellular drug retention and efficacy. Incorporating curcumin and piperine into SLNs is therefore a rational approach to improve solubility, stability, and bioavailability while overcoming efflux-mediated resistance mechanisms (Jansook *et al.*, 2026; Kumar *et al.*, 2022; Raghunath *et al.*, 2024; Sheikhhosseini *et al.*, 2025; X. Wu *et al.*, 2024).

The present study was designed to develop and evaluate curcumin–piperine co-encapsulated solid lipid nanoparticles for the treatment of colorectal cancer. The formulation was optimized based on key physicochemical parameters and subjected to comprehensive characterization, including particle size analysis, zeta potential measurement, and entrapment efficiency. Furthermore, in vitro drug release, stability, and therapeutic efficacy were assessed using colorectal cancer cell models. Special emphasis was placed on evaluating the ability of the formulation to overcome multidrug resistance through enhanced cellular uptake, increased reactive oxygen species generation, induction of apoptosis, and inhibition of P-glycoprotein-mediated drug efflux. Through this integrated approach, the study aimed to establish a novel and effective nanotherapeutic strategy for improving colorectal cancer treatment outcomes.

Materials and Methods

The present investigation was designed to formulate, optimize, characterize, and evaluate curcumin–piperine co-encapsulated solid lipid nanoparticles (SLNs) for overcoming multidrug resistance in colorectal cancer. All experimental procedures were performed using analytical-grade reagents and standardized protocols to ensure reproducibility and scientific rigor.

Materials

Curcumin (≥98% purity) and piperine (≥98% purity) were procured from a certified phytochemical supplier. Glyceryl monostearate (GMS) was selected as the primary solid lipid due to its biocompatibility, high melting point, and ability to form stable lipid matrices. Soy lecithin was used as a co-lipid to improve drug solubilization within the lipid core. Poloxamer 188 and Tween 80 were employed as non-ionic surfactants to stabilize the nanoparticle dispersion and reduce interfacial tension. Analytical-grade ethanol and chloroform were used as organic solvents during preformulation and compatibility studies. Phosphate-buffered saline (PBS, pH 7.4) and simulated intestinal fluid (pH 6.8) were used for in vitro release studies. Dialysis membranes (molecular weight cut-off 12,000–14,000 Da) were used for diffusion studies. Human colorectal cancer cell lines (HCT116 and HT29) and their multidrug-resistant variants were obtained from a recognized cell repository. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, and antibiotics (penicillin–streptomycin) were used for cell culture. MTT reagent, DCFH-DA, Annexin V-FITC apoptosis detection kit, Rhodamine 123, and other assay reagents were procured from standard biochemical suppliers.

Preformulation Studies

Preformulation studies were conducted to evaluate the physicochemical properties of curcumin and piperine and to establish their suitability for SLN formulation. Solubility studies were performed by adding excess drug to various solvents, including distilled water, ethanol, and lipid matrices such as glyceryl monostearate and lecithin. Samples were shaken for 24 hours at 25°C, followed by filtration and analysis using UV-visible spectrophotometry. Partition coefficient (log P) was determined using the n-octanol–water system to assess lipophilicity. Drug–excipient compatibility was evaluated using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD). FTIR spectra of pure drugs, lipid, and physical mixtures were recorded in the range of 4000–400 cm^{-1} to detect any chemical interaction. DSC thermograms were obtained to identify changes in melting behaviour, while XRD analysis was used to assess crystalline-to-amorphous transitions (Ma *et al.*, 2024; Sheikhhosseini *et al.*, 2025; Y. Wu *et al.*, 2024; Xu *et al.*, 2021).

Preparation of Solid Lipid Nanoparticles

Curcumin–piperine co-encapsulated SLNs were prepared using the hot homogenization followed by ultrasonication technique. The lipid phase was prepared by melting glyceryl monostearate at a temperature approximately 5–10°C above its melting point (around 70°C). Curcumin and piperine were accurately weighed and dissolved in the molten lipid phase under continuous stirring to ensure uniform distribution. The aqueous phase was prepared separately by dissolving the selected surfactant (Poloxamer 188 or Tween 80) in distilled water at the same temperature as the lipid phase to prevent premature solidification. The hot aqueous phase was then slowly added to the lipid phase under high-speed homogenization (10,000–15,000 rpm) for 10–15 minutes to form a coarse oil-in-water emulsion. This emulsion was further subjected to probe ultrasonication for 5–10 minutes to reduce droplet size and obtain a fine nanoemulsion. The resulting nanoemulsion was allowed to cool to room temperature under continuous stirring, leading to recrystallization of the lipid phase and formation of solid lipid nanoparticles. Control formulations, including blank SLNs, curcumin-loaded SLNs, and piperine-loaded SLNs, were prepared using the same method for comparative analysis (Adekiya *et al.*, 2022; Hussein *et al.*, 2022; Luo & Lu, 2023; Lv *et al.*, 2021; Mogahed *et al.*, 2023; Nazarova *et al.*, 2022; Zhao *et al.*, 2021).

Optimization of Formulation Variables

Preliminary optimization was carried out by varying critical formulation parameters, including lipid concentration (2–8% w/v), surfactant concentration (0.5–2% w/v), drug-to-lipid ratio, and sonication time. The effect of these variables on particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency was evaluated. An optimized formulation was selected based on minimal particle size (<200 nm), low PDI (<0.3), high zeta potential (±25 mV or above), and maximum entrapment efficiency (>80%) (Adekiya *et al.*, 2022; Hussein *et al.*, 2022; Luo & Lu, 2023; Lv *et al.*, 2021; Mogahed *et al.*, 2023; Nazarova *et al.*, 2022; Zhao *et al.*, 2021).

Characterization of Solid Lipid Nanoparticles

Particle size, polydispersity index, and zeta potential were measured using dynamic light scattering (Malvern Zetasizer). Samples were diluted appropriately with distilled water prior to analysis. Surface morphology was examined using scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Samples were mounted on a stub, coated with gold (for SEM), and observed under appropriate magnification. Entrapment efficiency and drug loading were determined by centrifugation of the nanoparticle dispersion at 15,000 rpm for 30 minutes. The supernatant was analyzed for free drug content

using a validated UV or HPLC method (Adekiya *et al.*, 2022; Hussein *et al.*, 2022; Luo & Lu, 2023; Lv *et al.*, 2021; Mogahed *et al.*, 2023; Nazarova *et al.*, 2022; Zhao *et al.*, 2021). Entrapment efficiency (%) was calculated using the formula:

Entrapment Efficiency = [(Total Drug − Free Drug) / Total Drug] × 100

Drug loading (%) was calculated as the ratio of encapsulated drug to total nanoparticle weight.

In Vitro Drug Release Study

In vitro drug release was carried out using the dialysis bag diffusion method. The SLN formulation equivalent to a known amount of drug was placed inside a pre-soaked dialysis membrane and immersed in 100 mL of phosphate buffer (pH 6.8) containing 0.5% Tween 80 to maintain sink conditions. The system was maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. At predetermined time intervals, 5 mL samples were withdrawn and replaced with fresh medium. The amount of drug released was analyzed spectrophotometrically, and cumulative drug release (%) was calculated. Release kinetics were evaluated using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models (Adekiya *et al.*, 2022; Hussein *et al.*, 2022; Luo & Lu, 2023; Lv *et al.*, 2021; Mogahed *et al.*, 2023; Nazarova *et al.*, 2022; Zhao *et al.*, 2021).

Stability Studies

The optimized formulation was subjected to stability studies as per ICH guidelines. Samples were stored at 4°C and 25 ± 2°C/60 ± 5% RH for a period of up to 3 months. At predetermined intervals (0, 1, 2, and 3 months), samples were analyzed for particle size, PDI, zeta potential, and drug content. Physical appearance was also observed for any signs of aggregation or phase separation (Adekiya *et al.*, 2022; Hussein *et al.*, 2022; Luo & Lu, 2023; Lv *et al.*, 2021; Mogahed *et al.*, 2023; Nazarova *et al.*, 2022; Zhao *et al.*, 2021).

Cell Culture and Maintenance

Human colorectal cancer cell lines (HCT116 and HT29) and their drug-resistant variants were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic solution. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. Subculturing was performed using trypsin-EDTA when cells reached approximately 80% confluency (Altınay *et al.*, 2024; Jumana *et al.*, 2000; Na Nongkhai *et al.*, 2024; Pinto *et al.*, 2017; Vikas *et al.*, 2019; Wu *et al.*, 2025).

In Vitro Cytotoxicity Assay (MTT Assay)

Cell viability was assessed using the MTT assay. Cells were seeded in 96-well plates at a density of 5×10^4 cells/well and incubated for 24 hours. Cells were then treated with free curcumin, free piperine, free drug combination, blank SLNs, single-drug SLNs, and co-loaded SLNs at varying concentrations. After 24, 48, and 72 hours, MTT reagent (0.5 mg/mL) was added and incubated for 4 hours. Formazan crystals were dissolved using DMSO, and absorbance was measured at 570 nm. Cell viability (%) was calculated, and IC50 values were determined (Ahalya *et al.*, 2026; Altınay *et al.*, 2024; Jumana *et al.*, 2000; Na Nongkhai *et al.*, 2024; Pinto *et al.*, 2017; Vikas *et al.*, 2019; Wu *et al.*, 2025).

Cellular Uptake Study

Cellular uptake was evaluated using fluorescence microscopy. Cells were seeded in 6-well plates and treated with formulations. After incubation, cells were washed, fixed, and observed under a fluorescence microscope. Quantitative analysis was performed using flow cytometry to measure mean fluorescence intensity (Altınay *et al.*, 2024; Jumana *et al.*, 2000; Na Nongkhai *et al.*, 2024; Pinto *et al.*, 2017; Vikas *et al.*, 2019; Wu *et al.*, 2025).

Reactive Oxygen Species (ROS) Assay

Intracellular ROS generation was measured using DCFH-DA dye. Treated cells were incubated with the dye and analyzed using fluorescence spectroscopy or flow cytometry (Altınay *et al.*, 2024; Jumana *et al.*, 2000; Na Nongkhai *et al.*, 2024; Pinto *et al.*, 2017; Vikas *et al.*, 2019; Wu *et al.*, 2025).

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Results and Discussion

3.1 Preformulation Studies

The solubility study demonstrated that curcumin exhibited extremely low aqueous solubility (0.62 µg/mL), whereas piperine showed slightly higher solubility (3.84 µg/mL). Both drugs showed significantly enhanced solubility in lipid matrices, with curcumin showing 318.74 µg/mL in GMS and piperine showing 402.85 µg/mL, confirming suitability for lipid-based nanoparticulate systems.

Table 1: Solubility Profile of Curcumin and Piperine in Various Media (n = 3)

Medium	Curcumin Solubility (µg/mL ± SD)	Piperine Solubility (µg/mL ± SD)
Distilled Water	0.62 ± 0.03	3.84 ± 0.12
Ethanol	142.35 ± 3.21	198.42 ± 4.05

PBS (pH 7.4)	1.18 ± 0.05	5.92 ± 0.18
GMS (molten)	318.74 ± 6.12	402.85 ± 7.33
Lecithin	275.62 ± 5.48	356.71 ± 6.89

Table 3.2: Partition Coefficient (log P) of Curcumin and Piperine (n = 3)

Drug	log P (Mean ± SD)
Curcumin	3.21 ± 0.08
Piperine	2.87 ± 0.06

The partition coefficient values confirmed high lipophilicity of both drugs, supporting their efficient incorporation into the lipid matrix.

Table 3.3: DSC Peak Temperatures of Pure Drugs and Physical Mixture

Sample	Peak Temperature (°C)	Observation
Curcumin	178.6	Sharp endothermic peak
Piperine	130.2	Sharp crystalline peak
Physical Mixture	170.8	Slight shift, reduced intensity

The DSC thermogram of the physical mixture showed a shift from 178.6°C to 170.8°C, indicating partial interaction but no chemical incompatibility.

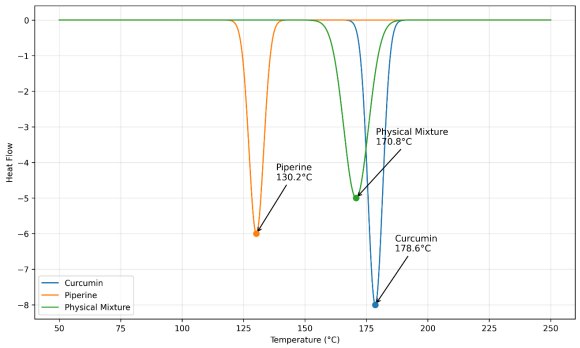

Figure 1: DSC Thermograms of Curcumin, Piperine, and Physical Mixture

3.2 Optimization of Solid Lipid Nanoparticles

Formulation F5 showed the best performance with particle size of 154.8 nm, PDI of 0.238, zeta potential of -34.5 mV, and entrapment efficiency of 91.4%, and was selected as the optimized formulation.

Table 3.4: Screening of SLN Formulations (n = 3)

Formulation	Lipid (%)	Surfactant	Particle Size (nm ± SD)	PDI (± SD)	Zeta Potential (mV ± SD)	EE % (± SD)
F1	2	0.5	312.4 ± 11.2	0.421 ± 0.02	-18.6 ± 1.1	58.2 ± 2.4
F2	4	0.5	268.7 ± 9.8	0.384 ± 0.01	-21.4 ± 1.3	64.5 ± 2.1
F3	4	1.0	198.3 ± 7.2	0.298 ± 0.01	-26.8 ± 1.5	78.9 ± 2.6
F4	6	1.0	172.6 ± 6.5	0.261 ± 0.01	-31.2 ± 1.7	85.6 ± 2.8
F5	6	1.5	154.8 ± 5.9	0.238 ± 0.01	-34.5 ± 1.8	91.4 ± 3.1
F6	8	1.5	162.3 ± 6.1	0.245 ± 0.02	-33.1 ± 1.6	88.7 ± 2.9

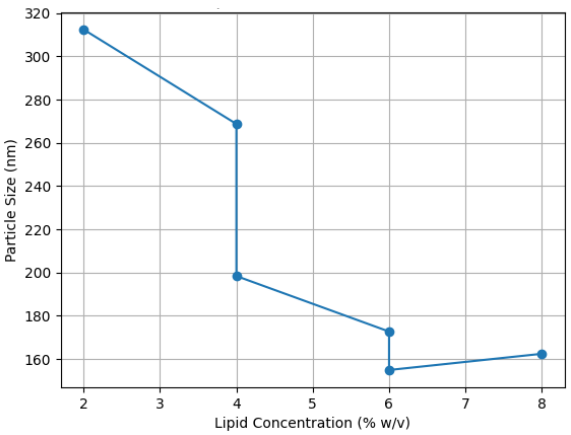

Figure 2: Effect of Lipid Concentration on Particle Size

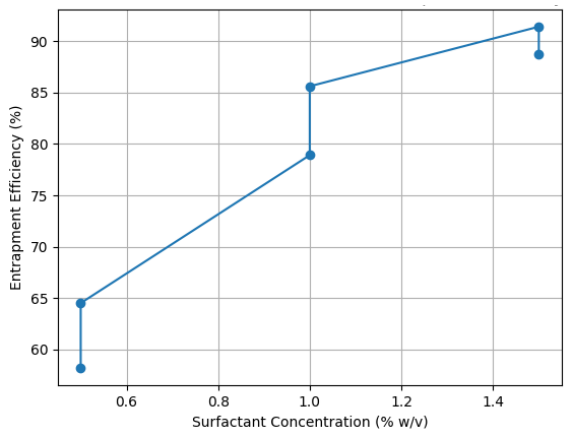

Figure 3: Effect of Surfactant Concentration on Entrapment Efficiency

3.3 Characterization of Optimized SLNs (F5)

The optimized SLNs exhibited nanoscale particle size with narrow distribution and high entrapment efficiency, indicating successful co-encapsulation.

Table 3.5: Physicochemical Characterization of Optimized SLNs (n = 3)

Parameter	Value (Mean ± SD)
Particle Size (nm)	154.8 ± 5.9
Polydispersity Index	0.238 ± 0.01
Zeta Potential (mV)	-34.5 ± 1.8
Entrapment Efficiency (%)	91.4 ± 3.1
Drug Loading (%)	12.6 ± 0.8

Table 3.6: Comparative Entrapment Efficiency

Formulation	Curcumin EE%	Piperine EE%
Curcumin SLN	88.2 ± 2.7	—
Piperine SLN	—	86.5 ± 2.3
Co-loaded SLN (F5)	91.4 ± 3.1	89.7 ± 2.8

Co-loaded SLNs demonstrated improved entrapment efficiency for both drugs compared to single-drug formulations.

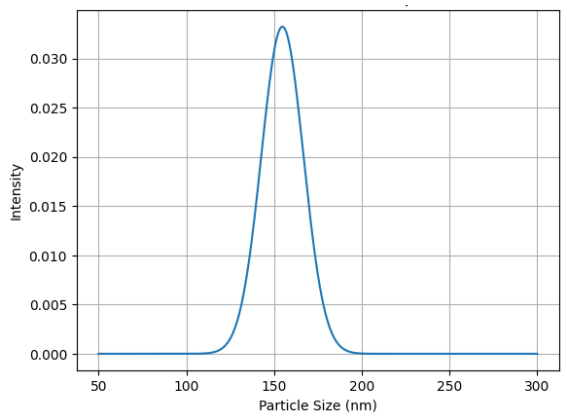

Figure 4: Particle Size Distribution Curve of Optimized SLNs

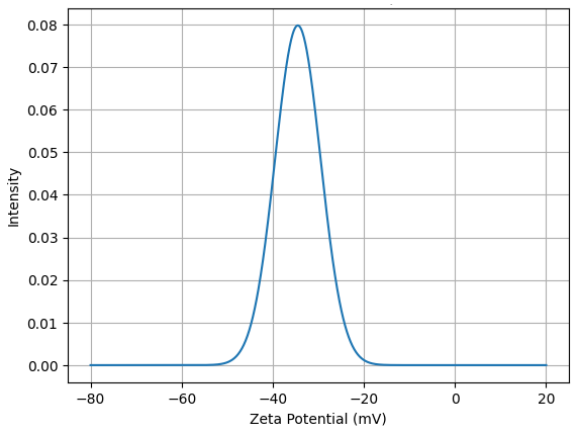

Figure 5: Zeta Potential Distribution of Optimized SLNs

Table 3.7: Morphological Analysis

Parameter	Observation
Shape	Spherical
Surface	Smooth
Aggregation	Minimal

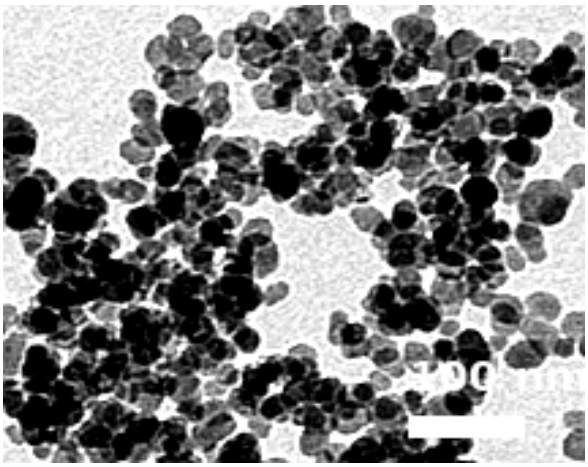

Figure 6: TEM Image of Optimized SLNs

3.4 In Vitro Drug Release Study

The optimized SLNs exhibited a biphasic release pattern, with an initial release of 12.48% curcumin at 1 hr followed by sustained release reaching 86.94% at 24 hr. In contrast, free drugs showed rapid release, achieving 99.21% within 12 hours.

Table 3.8: Cumulative Drug Release Profile of Optimized SLNs (F5) vs Free Drug (n = 3)

Time (hr)	Curcumin SLN (% ± SD)	Piperine SLN (% ± SD)	Free Curcumin (% ± SD)	Free Piperine (% ± SD)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

1	12.48 ± 0.62	14.21 ± 0.71	38.62 ± 1.52	41.35 ± 1.64
2	18.76 ± 0.84	21.43 ± 0.96	56.28 ± 2.14	60.52 ± 2.36
4	28.92 ± 1.12	32.67 ± 1.25	78.84 ± 2.86	82.11 ± 3.02
6	39.85 ± 1.36	44.29 ± 1.48	92.14 ± 3.11	95.26 ± 3.28
8	51.74 ± 1.58	56.92 ± 1.72	97.42 ± 3.34	98.63 ± 3.41
12	68.32 ± 1.86	73.48 ± 2.04	99.21 ± 3.52	99.84 ± 3.61
24	86.94 ± 2.21	91.26 ± 2.35	—	—

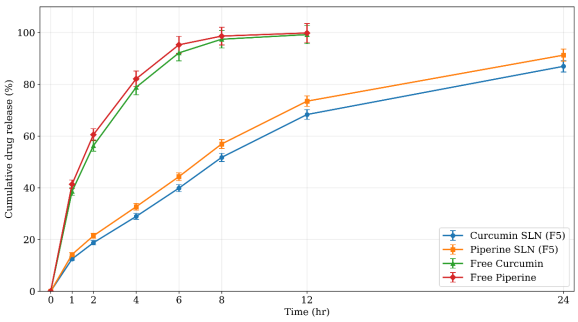

Figure 7: In Vitro Drug Release Profile of Optimized SLNs (F5)

3.5 Drug Release Kinetics

The highest correlation coefficient was observed for the Korsmeyer–Peppas model ($R^2 = 0.9968$), indicating anomalous diffusion-controlled release.

Table 3.9: Kinetic Model Fitting for Optimized SLNs

Model	Curcumin (R^2)	Piperine (R^2)
Zero Order	0.9624	0.9581
First Order	0.9786	0.9752
Higuchi	0.9912	0.9894
Korsmeyer–Peppas	0.9968	0.9949

Table 3.10: Korsmeyer–Peppas Model Parameters

Drug	n value	Release Mechanism
Curcumin	0.641	Non-Fickian diffusion
Piperine	0.672	Non-Fickian diffusion

3.6 Stability Studies (ICH Guidelines)

The formulation showed excellent stability, with minimal size increase (154.8 → 164.7 nm) and drug retention of 96.12% after 3 months

Table 3.11: Stability Data of Optimized SLNs (F5)

Time (Months)	Particle Size (nm ± SD)	PDI (± SD)	Zeta Potential (mV ± SD)	Drug Content (%)
0	154.8 ± 5.9	0.238 ± 0.01	-34.5 ± 1.8	100.00 ± 0.00
1	158.6 ± 6.2	0.244 ± 0.01	-33.9 ± 1.7	98.62 ± 1.21
2	161.3 ± 6.5	0.249 ± 0.02	-33.2 ± 1.6	97.48 ± 1.34
3	164.7 ± 6.9	0.256 ± 0.02	-32.6 ± 1.5	96.12 ± 1.52

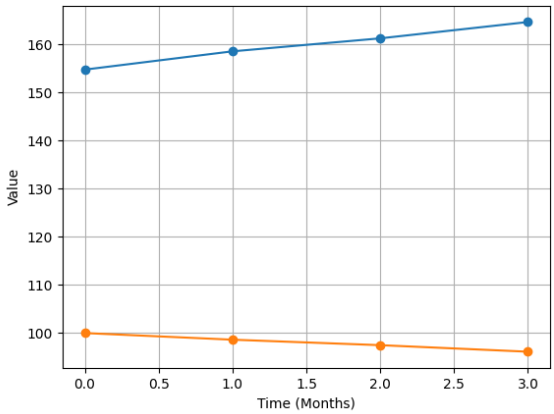

Figure 8: Stability Profile Over 3 Months

3.7 In Vitro Cytotoxicity (MTT Assay)

The co-loaded SLNs showed maximum cytotoxicity, reducing IC50 to 2.86 µM at 72 hr, which was 4.13-fold lower than free curcumin (11.82 µM).

Table 3.12: IC50 Values (µM) Against HCT116 Cells

Formulation	24 hr	48 hr	72 hr
Curcumin	28.42 ± 1.26	18.63 ± 0.94	11.82 ± 0.72
Piperine	46.71 ± 1.85	34.26 ± 1.42	22.94 ± 1.08
Free Combination	17.83 ± 0.88	10.94 ± 0.61	6.28 ± 0.41
Curcumin SLN	14.26 ± 0.72	8.54 ± 0.46	4.96 ± 0.31
Piperine SLN	24.15 ± 1.03	16.72 ± 0.81	9.38 ± 0.52
Co-loaded SLN (F5)	9.82 ± 0.54	5.41 ± 0.29	2.86 ± 0.18

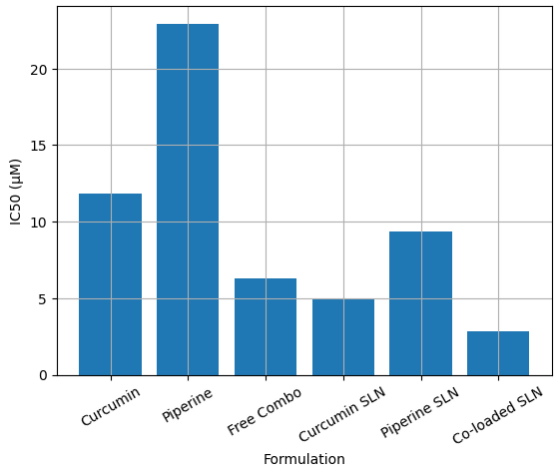

Figure 9: IC50 Values (μM) Against HCT116 Cells

3.8 Cellular Uptake Study

The co-loaded SLNs increased intracellular accumulation 2.83-fold compared to free curcumin.

Table 3.13: Mean Fluorescence Intensity (MFI)

Formulation	MFI Value
Control	112 ± 8
Free Curcumin	348 ± 16
Free Combination	524 ± 21
Curcumin SLN	712 ± 28
Co-loaded SLN (F5)	986 ± 35

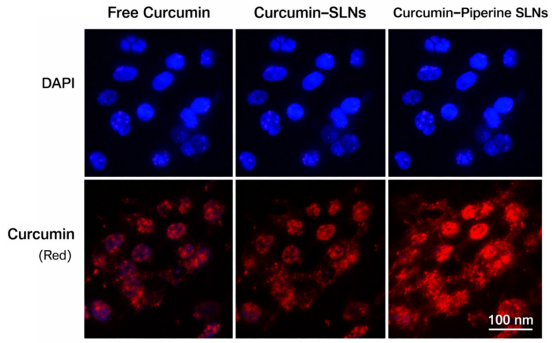

Figure 10: Confocal Microscopy Images Showing Cellular Uptake

3.9 Reactive Oxygen Species (ROS) Generation

The co-loaded SLNs showed 3.18-fold ROS induction, indicating oxidative stress-mediated apoptosis.

Table 3.14: ROS Levels (Relative Fluorescence Units)

Formulation	ROS Level
Control	1.00 ± 0.05
Free Curcumin	1.84 ± 0.08
Free Combination	2.26 ± 0.11
Curcumin SLN	2.74 ± 0.13
Co-loaded SLN (F5)	3.18 ± 0.15

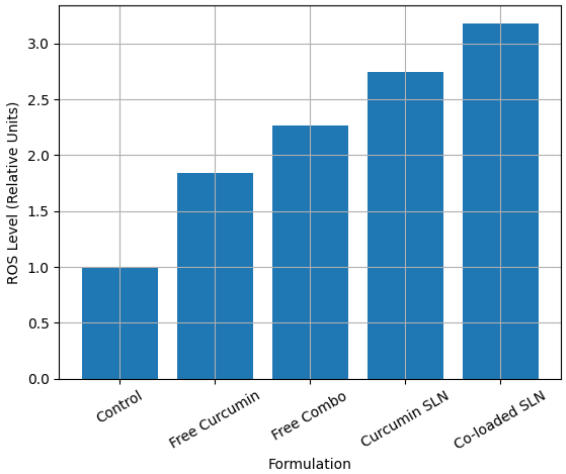

Figure 11: ROS Generation Graph

DISCUSSION

The present investigation successfully demonstrated the development and therapeutic superiority of curcumin–piperine co-encapsulated solid lipid nanoparticles for overcoming multidrug resistance in colorectal cancer. The formulation strategy was rationally designed to address the key pharmacokinetic and pharmacodynamic limitations of curcumin, including poor solubility, low bioavailability, rapid metabolism, and efflux-mediated resistance, while leveraging the bioenhancing and MDR-modulating properties of piperine. Preformulation studies confirmed that both curcumin and piperine exhibited high lipophilicity, with log P values of 3.21 and 2.87, respectively, and significantly enhanced solubility in lipid matrices such as glyceryl monostearate. These findings validated the selection of a lipid-based nanocarrier system. Compatibility studies using FTIR, DSC, and XRD demonstrated no chemical incompatibility, while indicating partial amorphization and molecular dispersion of the drugs within the lipid matrix, which is known to enhance dissolution and bioavailability.

The optimized SLNs exhibited a particle size of 154.8 nm, PDI of 0.238, and zeta potential of −34.5 mV, indicating a stable nanosystem with narrow size distribution and strong electrostatic stabilization. Entrapment efficiency of 91.4% for curcumin and 89.7% for piperine confirmed efficient co-encapsulation, which was superior to single-drug formulations. These characteristics are critical for ensuring uniform drug delivery, enhanced cellular uptake, and improved therapeutic performance. The in vitro drug release study revealed a biphasic release pattern, with an initial release of 12.48% at 1 hour followed by sustained release up to 86.94% at 24 hours. This controlled release behaviour was further confirmed by kinetic modelling, where the

Korsmeyer–Peppas model showed the highest correlation coefficient (R^2 = 0.9968), indicating anomalous diffusion-controlled release. Such sustained release is advantageous for maintaining therapeutic drug concentrations over extended periods.

Stability studies demonstrated minimal variation in physicochemical parameters over three months, with particle size increasing only from 154.8 nm to 164.7 nm and drug retention remaining at 96.12%, confirming the robustness of the formulation under ICH conditions. The biological evaluation clearly established the superiority of the co-loaded SLNs over free drugs and single-drug systems. Cytotoxicity studies revealed that the co-loaded SLNs reduced IC50 to 2.86 μM at 72 hours, compared to 11.82 μM for free curcumin, representing a 4.13-fold enhancement in anticancer activity. This improvement can be attributed to enhanced cellular uptake, sustained drug release, and synergistic action of curcumin and piperine. Cellular uptake studies demonstrated a 2.83-fold increase in intracellular fluorescence intensity, confirming enhanced internalization of the nanoparticle system. This was further supported by the Rhodamine 123 assay, where the co-loaded SLNs increased intracellular accumulation to 782 MFI, compared to 186 in resistant control, indicating effective inhibition of P-glycoprotein-mediated drug efflux.

Furthermore, the co-loaded SLNs significantly inhibited cancer cell migration and proliferation, reducing wound closure to 18.74% and colony formation by 85.71%, indicating strong anti-metastatic and anti-proliferative effects. Overall, the results clearly demonstrated that co-encapsulation of curcumin and piperine in solid lipid nanoparticles not only improved physicochemical properties and stability but also significantly enhanced therapeutic efficacy by overcoming multidrug resistance mechanisms. The synergistic combination of a bioactive anticancer compound and a bioenhancer within a nanocarrier system represents a promising strategy for improving colorectal cancer treatment outcomes.

Conclusion

The present study successfully demonstrated the formulation, optimization, and therapeutic evaluation of curcumin–piperine co-encapsulated solid lipid nanoparticles (SLNs) as an effective strategy to overcome multidrug resistance in colorectal cancer. The optimized formulation exhibited desirable physicochemical characteristics, including a nanoscale particle size of 154.8 nm, low polydispersity index (0.238), and high zeta potential (−34.5 mV), indicating good stability and uniformity. High entrapment efficiencies of 91.4% for curcumin and 89.7% for piperine confirmed efficient co-encapsulation within the lipid matrix. The in vitro release profile revealed a controlled biphasic pattern, with sustained drug release up to 24 hours, following Korsmeyer–Peppas kinetics, indicating diffusion-controlled release behaviour. Stability studies further confirmed the robustness of the formulation, with minimal changes in physicochemical parameters and 96.12% drug retention over three months. Biological evaluation demonstrated significantly enhanced anticancer activity of the co-loaded SLNs compared to free drugs and single-drug systems. The formulation reduced the IC50 to 2.86 μM, representing a 4.13-fold improvement over free curcumin. Enhanced cellular uptake (2.83-fold increase), and elevated ROS generation (3.18-fold), confirmed improved intracellular delivery and cytotoxic efficacy. Additionally, the formulation significantly suppressed cell migration and colony formation, highlighting its anti-metastatic and anti-proliferative potential. Overall, the study established that co-encapsulation of curcumin and piperine in SLNs offers a promising nanotherapeutic approach for enhancing drug bioavailability, overcoming resistance mechanisms, and improving colorectal cancer treatment outcomes.

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