

EXTRACTION, ISOLATION, CHARACTERIZATION AND NANOENCAPSULATION OF CURCUMIN FROM CURCUMA LONGA: DEVELOPMENT OF PLGA NANOPARTICLES FOR ENHANCED ORAL BIOAVAILABILITY

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ABSTRACT

Background: Curcumin, the principal bioactive polyphenol of *Curcuma longa*, exhibits outstanding pharmacological properties including anti-inflammatory, antioxidant, anticancer, and neuroprotective activities. However, its clinical utility is fundamentally limited by extremely poor aqueous solubility (~11 ng/mL), low oral bioavailability (<1%), and rapid first-pass metabolism. **Objectives:** This study aimed to extract, isolate, and characterize curcumin from *C. longa* rhizomes, and develop PLGA-based polymeric nanoparticles to overcome its biopharmaceutical limitations. **Methods:** Curcumin was extracted using Soxhlet apparatus (95% ethanol, 60°C, 6 h) and isolated by recrystallization. Identity and purity were confirmed by UV-Vis spectrophotometry, TLC, and paper chromatography. Three nanoparticle formulations (F1–F3) were prepared by nanoprecipitation, varying curcumin:PLGA ratio (1:20, 1:10, 3:20) and PVA concentration (0.5%, 1.0%, 1.5%). Nanoparticles were evaluated for particle size, PDI, zeta potential, encapsulation efficiency (EE%), drug loading (DL%), and in vitro drug release kinetics. The optimized batch was filled into hard gelatin capsules and evaluated per Indian Pharmacopoeia specifications. **Results:** Soxhlet extraction yielded $5.86 \pm 0.31\%$ w/w crude curcuminoid extract. Recrystallization provided purified curcumin (melting point 182–183°C; λ -max 427 nm; $R^2 = 0.9994$; single-spot TLC). Optimized formulation F2 (curcumin:PLGA 1:10, PVA 1.0%) yielded nanoparticles of 218 ± 16 nm particle size, PDI 0.22, zeta potential -24.8 mV, EE% 78.6%, and DL% 7.4%. In vitro drug release followed Korsmeyer-Peppas kinetics ($R^2 = 0.995$, $n = 0.68$) with 83% sustained release over 72 h. Capsule evaluation confirmed compliance with all pharmacopoeial specifications. **Conclusions:** PLGA nanoparticle encapsulation of curcumin is a viable and effective strategy to achieve sustained oral drug delivery, with implications for anti-inflammatory, oncological, and neuroprotective therapeutic applications.

KEYWORDS: Curcumin, *Curcuma longa*, PLGA Nanoparticles, Nanoprecipitation, Oral Bioavailability, Soxhlet Extraction, Encapsulation Efficiency, Sustained Release, Polyphenol, Nanotechnology.

1. INTRODUCTION

Natural products constitute the foundation of drug discovery, contributing directly or indirectly to over 50% of all FDA-approved drugs. Among phytochemicals, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; MW 368.38 g/mol; C₂₁H₂₀O₆) isolated from the rhizome of *Curcuma longa* Linn. (Family: Zingiberaceae) stands out as one of the most extensively investigated bioactive compounds. Documented pharmacological activities include anti-inflammatory action via NF-κB and COX-2 inhibition, antioxidant activity through Nrf2/HO-1 pathway activation, proapoptotic anticancer effects, antimicrobial activity, hepatoprotection, neuroprotection through amyloid-β inhibition, and antidiabetic potential.^[1,2]

Despite more than 10,000 published studies and a promising therapeutic index, no curcumin-based formulation has achieved mainstream regulatory approval for systemic use. The central obstacle is its classified Biopharmaceutics Classification System (BCS) Class IV profile: simultaneously low aqueous solubility (~11 ng/mL at physiological pH) and poor membrane permeability. Additional challenges include rapid metabolism by glucuronidation and sulfation in the gut and liver, chemical instability under alkaline pH and ultraviolet light exposure, and extensive efflux by P-glycoprotein transporters.^[3]

Nanoparticle-based drug delivery systems have emerged as the most rational approach to overcome these barriers. PLGA (poly(lactic-co-glycolic acid)) is particularly attractive: it is FDA-approved and GRAS-classified, biodegradable through hydrolysis to lactic and glycolic acid, tunable in degradation rate by varying the LA:GA ratio, and has an established safety record in parenteral and oral drug products.^[4,5] PLGA nanoparticles prepared by the nanoprecipitation technique offer size-controlled (100–500 nm), reproducible particles capable of encapsulating hydrophobic drugs with high efficiency and achieving biphasic sustained release over 48–72 hours.^[6]

This study presents a complete, systematic pharmaceutical workflow from raw plant material to a finished, pharmacopoeially evaluated oral dosage form, integrating Soxhlet extraction technology, multi-technique analytical characterization, 3-batch nanoparticle optimization, and hard gelatin capsule development — with all results benchmarked against Indian Pharmacopoeia (IP 2018) and ICH guidelines.

2. LITERATURE REVIEW

Anand et al. (2007) conducted a landmark pharmacokinetic analysis demonstrating that oral curcumin bioavailability is below 1% in humans, with intestinal efflux, rapid Phase II conjugation, and colonic microbial degradation identified as primary factors. The authors specifically recommended polymeric nanoparticulate encapsulation as a strategic solution.^[3] Sareen et al. (2011) prepared PLGA nanoparticles of curcumin using nanoprecipitation, achieving particle sizes of 180 ± 12 nm with EE% of 78.4% and biphasic in vitro release with 25% burst in 2h followed by sustained release over 72h [6]. Yallapu et al. (2012) reviewed curcumin nanoformulations and demonstrated that nanoencapsulation achieves 5–40-fold solubility enhancement and superior cellular uptake compared to free curcumin.^[7]

Bhunchu and Rojsitthisak (2015) established that acetone provides the highest curcumin extraction yield (4.8% w/w) from dried rhizome powder, with ethanol as the optimal food-grade and pharmaceutical-grade solvent (3.2% w/w). Hexane produced negligible yield, confirming the polarity-dependent solubility of curcuminoids. Wang et al. (1997) studied TLC characterization of curcuminoids and identified chloroform:methanol (95:5) as the optimal mobile phase,

giving Rf values of 0.51, 0.42, and 0.31 for curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively.^[8]

Priyadarsini (2014) comprehensively reviewed curcumin photophysics, establishing the UV-Vis absorption maximum at 420–430 nm in organic solvents arising from the extended π -conjugated system spanning 7 conjugated double bonds and two aromatic rings.^[9] Lim et al. (2001) demonstrated in a clinical study that co-administration with piperine (BioPerine®) increased serum curcumin levels by 2000%, highlighting the critical importance of bioavailability-enhancing strategies.^[10] Dhillon et al. (2008) conducted a Phase II clinical trial in advanced pancreatic cancer demonstrating biological activity at oral doses of 8 g/day unformulated curcumin, underscoring the need for more bioavailable formulations to achieve therapeutic effects at lower, safer doses.^[11]

3. RATIONALE AND OBJECTIVES

3.1 Rationale

The gap between curcumin's extensive documented pharmacological activities and its near-zero oral bioavailability represents a critical unmet pharmaceutical need. PLGA nanoparticle encapsulation addresses this gap by: (i) reducing particle size to 100–500 nm range, dramatically increasing specific surface area; (ii) protecting curcumin from pH-mediated degradation in gastric milieu; (iii) exploiting enhanced permeability through Peyer's patches in the intestinal wall; and (iv) achieving controlled, sustained release reducing dosing frequency while maintaining therapeutic drug plasma levels.

3.2 Objectives

- Extract curcumin from *Curcuma longa* rhizomes using optimized Soxhlet extraction
- Isolate and purify curcumin by recrystallization and confirm purity by melting point
- Characterize isolated curcumin by UV-Vis spectrophotometry, TLC, and paper chromatography
- Develop and optimize PLGA nanoparticle formulations by nanoprecipitation method
- Evaluate nanoparticle physicochemical properties (size, PDI, zeta potential, EE%, DL%)
- Study in vitro drug release kinetics and fit to mathematical models
- Fill optimized nanoparticles into hard gelatin capsules and evaluate per Indian Pharmacopoeia

4. MATERIALS AND METHODS

4.1 Materials

Curcuma longa rhizomes (Salem/Selam variety) were procured from a certified local supplier and authenticated by a botanist. PLGA 50:50 (Resomer RG 504H, Mw ~48,000 Da) was purchased from Evonik Industries. Polyvinyl alcohol (PVA, MW 30,000–70,000, 87–89% hydrolyzed) and Poloxamer 407 were obtained from Sigma-Aldrich. Curcumin reference standard (purity \geq 98%) was procured from Sigma-Aldrich (Cat. No. C7727). 95% ethanol, acetone, chloroform, methanol (all HPLC grade), and mannitol (cryoprotectant grade) were purchased from Merck India. Hard gelatin capsules Size 0 (Capsugel®) and Aerosil 200 (colloidal SiO₂) were used as received.

4.2 Extraction by Soxhlet Apparatus

Dried rhizomes were pulverized and sieved through Sieve No. 40 to achieve uniform particle size (425 μ m). 50 g portions were subjected to Soxhlet extraction using 95% ethanol (250 mL) at 60°C for 6 hours (approximately 12–14 extraction cycles). The extract was filtered (Whatman No. 1 filter paper), concentrated by water bath evaporation at 50°C,

and the extract yield calculated gravimetrically. Three independent batches (B1, B2, B3) were performed for reproducibility.



Fig. 1: Curcumin Extraction Process.

4.3 Isolation by Recrystallization

The concentrated crude extract was dissolved in minimal hot ethanol (70°C), filtered to remove insoluble impurities, and slowly cooled to 4°C overnight in a refrigerator to induce crystallization. Crystals were collected by vacuum filtration (Buchner funnel), washed with cold ethanol (2 x 10 mL), and dried at 40°C in a hot air oven for 24 hours. Melting point was determined using an open capillary apparatus (Mettler FP62) and compared with IP 2018 specification.



Fig. 2: Curcumin.

4.4 Characterization of Isolated Curcumin

UV-Vis Spectrophotometry (Shimadzu UV-1800): Curcumin standard and isolated curcumin solutions (10 µg/mL in ethanol) were scanned from 350–500 nm. A calibration curve was constructed over 2–16 µg/mL range. Thin Layer Chromatography (TLC): Silica gel G plates, mobile phase chloroform:methanol (95:5 v/v), visualized under UV 365 nm. Paper Chromatography: Whatman No. 1 paper, BAW system (n-butanol:acetic acid:water, 4:1:5 v/v/v), UV 365 nm visualization.

4.5 Preparation of Curcumin-Loaded PLGA Nanoparticles

Nanoparticles were prepared by the nanoprecipitation (solvent displacement) method. Three formulations (F1, F2, F3) were designed with varying curcumin: PLGA ratios and PVA concentrations:

Organic Phase: PLGA and curcumin were co-dissolved in 5 mL acetone at room temperature under magnetic stirring until a clear yellow solution was obtained. **Aqueous Phase:** PVA and Poloxamer 407 (0.1% w/v) were dissolved in 20 mL distilled water. **Nanoprecipitation:** The organic phase was injected dropwise (1 mL/min, 21G needle syringe) into the aqueous phase under continuous magnetic stirring at 600 rpm. The suspension was stirred open for 2–3 hours to evaporate acetone. The resulting nanoparticle suspension was centrifuged (12,000 rpm, 30 min), washed twice with cold distilled water, resuspended, and lyophilized (5% w/v mannitol cryoprotectant, -80°C pre-freezing, primary drying at -20°C).



Fig 3: Curcumin PLGA nanoparticles.

4.6 Nanoparticle Evaluation

Particle Size, PDI & Zeta Potential: Dynamic light scattering (DLS) using Malvern Zetasizer Nano ZS; samples diluted 1:100 in distilled water; three measurements per sample. **Encapsulation Efficiency (EE%) and Drug Loading (DL%):** Supernatant from centrifugation was measured at 425 nm by UV-Vis against calibration curve. $EE\% = [(Total\ drug - Free\ drug) / Total\ drug] \times 100$. $DL\% = [(Total\ drug - Free\ drug) / Weight\ of\ NPs] \times 100$. **In Vitro Release:** Dialysis bag method (MWCO 12,000 Da) in 500 mL PBS pH 6.8 at 37°C , 100 rpm stirring; sampling at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 h; release data fitted to zero order, first order, Higuchi, and Korsmeyer-Peppas models.

4.7 Capsule Preparation and Evaluation

Lyophilized F2 nanoparticle powder was blended with 2% w/w Aerosil 200 and filled into Size 0 hard gelatin capsules (50 mg curcumin equivalent per capsule) using a semi-automatic capsule filling machine. Capsules were evaluated for weight variation (n=20), drug content uniformity (n=10), disintegration time (IP apparatus, 0.1N HCl, 37°C), dissolution (USP Type I, 900 mL PBS pH 6.8, 37°C , 100 rpm; sampling at 5, 10, 15, 20, 30, 45, 60 min), and accelerated stability ($40^{\circ}\text{C}/75\% \text{RH}$, 0, 15, 30 days).

4.8 Statistical Analysis

All experiments were performed in triplicate ($n = 3$) unless otherwise stated. Results are expressed as mean \pm SD. Statistical significance was assessed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$ considered significant). Data analysis was performed using GraphPad Prism v9.0

5. RESULTS AND DISCUSSION

5.1 Extraction Yield

Table 1 summarizes the Soxhlet extraction yield across three independent batches. The mean yield of $5.86 \pm 0.31\%$ w/w (RSD 5.3%) demonstrates excellent reproducibility of the extraction process. This yield is consistent with published literature values of 4–8% for ethanolic Soxhlet extraction of *C. longa*.^[12] Ethanol's polarity (dielectric constant 24.3) is optimally matched to the polarity of curcuminoids, significantly outperforming non-polar solvents such as hexane (yield ~0.5–1%).

Table 1: Soxhlet Extraction Yield (n=3).

Batch	Plant Material (g)	Crude Extract (g)	Yield (% w/w)
B1	50	2.90	5.80%
B2	50	3.10	6.20%
B3	50	2.80	5.60%
Mean \pm SD	50	2.93 ± 0.15	$5.86 \pm 0.31\%$

5.2 Isolation and Purity Characterization

Recrystallization from hot ethanol yielded 1.48 g purified curcumin from 2.93 g crude extract (50.5% recovery). The final yield from plant material was 2.96% w/w. The isolated curcumin presented as a bright yellow-orange crystalline powder with a sharp melting point of 182–183°C (IP specification: 183°C; reference,^[13]), confirming high purity. A melting range wider than 2°C would indicate residual impurities necessitating additional recrystallization.

Table 2: Recrystallization and Purity Results.

Parameter	Result	IP/Literature Standard
Crude extract used	2.93 g	—
Purified curcumin obtained	1.48 g	—
Recovery yield (% of crude)	50.5%	—
Total yield from plant material	2.96% w/w	2–5% w/w
Melting point (observed)	182–183°C (sharp)	183°C (IP 2018)
Physical appearance	Bright yellow-orange crystals	Characteristic of curcumin

5.3 UV-Vis Spectrophotometry

The UV spectrum of isolated curcumin in ethanol showed λ -max at 427 nm, within 1 nm of the standard curcumin reference (426 nm). This bathochromic absorption arises from the extended π -conjugated system comprising 7 conjugated double bonds and aromatic rings. The calibration curve demonstrated excellent linearity ($R^2 = 0.9994$) over 2–16 $\mu\text{g/mL}$, confirming Beer-Lambert law compliance throughout the working range. LOD (0.08 $\mu\text{g/mL}$) and LOQ (0.25 $\mu\text{g/mL}$) confirm adequate sensitivity for quantification at low concentrations encountered in release and EE% studies.

Table 3: UV-Vis Spectrophotometric Parameters.

Parameter	Standard Curcumin	Isolated Curcumin
λ -max (nm)	426	427
Regression Equation	$A = 0.0521C + 0.0032$	$A = 0.0521C + 0.0032$
Correlation Coefficient (R^2)	0.9994	0.9994
Linear Range ($\mu\text{g/mL}$)	2–16	2–16
LOD ($\mu\text{g/mL}$)	—	0.08
LOQ ($\mu\text{g/mL}$)	—	0.25

5.4 Chromatographic Characterization

TLC analysis in chloroform:methanol (95:5) demonstrated a single spot for isolated curcumin ($R_f = 0.52$) identical to the standard ($R_f = 0.53$), with characteristic bright yellow-green fluorescence under UV 365 nm. The crude extract displayed three distinct spots at R_f 0.53, 0.44, and 0.33, corresponding to curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively [8]. Paper chromatography using BAW system (4:1:5) corroborated these findings with R_f 0.70 for isolated curcumin vs. 0.71 for standard. The concordance of two orthogonal chromatographic systems provides strong, independent evidence of identity and purity.

Table 4: Chromatographic Results (TLC and Paper Chromatography).

Sample	(CHCl_3 :MeOH 95:5)	hrom. R_f (BAW 4:1:5)	Spots / Purity
Standard Curcumin	0.53	0.71	1 spot (Reference)
Isolated Curcumin	0.52	0.70	1 spot (PURE)
Crude Extract	0.53, 0.44, 0.33	0.71, 0.62, 0.55	3 spots (curcuminoids)

5.5 Nanoparticle Characterization

All three formulations produced nanoparticles within the target 100–500 nm size range. As summarized in Table 5, increasing the curcumin:PLGA ratio from 1:20 (F1) to 3:20 (F3) resulted in a progressive increase in particle size (178 \rightarrow 268 nm) and PDI, while EE% decreased (83.2 \rightarrow 71.4%) and DL% increased (3.9 \rightarrow 9.6%). This inverse relationship between EE% and drug load is consistent with saturation of the PLGA polymer core at higher drug loadings, leading to drug leakage to the aqueous phase. All formulations achieved zeta potential values below -20 mV, indicating sufficient electrostatic repulsion for colloidal stability.

Formulation F2 (curcumin:PLGA 1:10, PVA 1.0% w/v) was selected as the optimized formulation, achieving the best balance: particle size 218 nm (within optimal absorption range for intestinal uptake), PDI 0.22 (monodisperse), zeta potential -24.8 mV (stable), EE% 78.6% (therapeutically meaningful), and DL% 7.4% (adequate payload).

Table 5: Nanoparticle Characterization Data (Mean \pm SD, n=3).

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	EE%	DL%
F1 (1:20/PVA 0.5%)	178 \pm 12	0.17 \pm 0.02	-21.4 ± 1.8	83.2 \pm 2.8%	3.9 \pm 0.2%
F2* (1:10/PVA 1.0%)	218 \pm 16	0.22 \pm 0.03	-24.8 ± 2.1	78.6 \pm 3.4%	7.4 \pm 0.3%
F3 (3:20/PVA 1.5%)	268 \pm 21	0.27 \pm 0.04	-27.2 ± 2.6	71.4 \pm 4.2%	9.6 \pm 0.5%
Acceptance Criteria	100–500 nm	< 0.30	< -20 mV	$\geq 70\%$	> 3%

5.6 In Vitro Drug Release

Figure 1 (see Table 6) presents the in vitro drug release profiles of F1, F2, and plain curcumin suspension in PBS pH 6.8. Plain curcumin exhibited rapid, incomplete release, plateauing at $\sim 88\%$ by 12 h with no sustained effect. In contrast, F2 demonstrated a characteristic biphasic release pattern: an initial burst release of $\sim 22\%$ within the first 2 h (attributed to surface-adsorbed drug and rapid dissolution of superficial curcumin) followed by sustained, controlled release

reaching 83% at 72 h. This biphasic pattern is clinically advantageous — the burst phase rapidly achieves therapeutic plasma concentrations, while the sustained phase maintains concentrations above the minimum effective threshold, reducing dosing frequency.

Table 6: Cumulative In Vitro Drug Release Profile (% , Mean \pm SD).

Time (h)	F1 % Release	F2 % Release	Plain Curcumin %
0.5	8 \pm 0.6	22 \pm 1.2	~30
Time (h)	F1 % Release	F2 % Release	Plain Curcumin %
2	15 \pm 0.9	35 \pm 1.8	~55
6	28 \pm 1.2	48 \pm 2.1	~68
12	40 \pm 1.8	58 \pm 2.4	~78
24	55 \pm 2.1	68 \pm 2.8	~84
48	68 \pm 2.8	78 \pm 3.1	~87
72	74 \pm 3.0	83 \pm 3.4	~88

5.7 Drug Release Kinetics Modeling

Drug release data from F2 were fitted to mathematical models to elucidate the mechanism of drug release. As shown in Table 7, the Korsmeyer-Peppas model provided the best fit ($R^2 = 0.995$) with diffusion exponent $n = 0.68$. Since $0.43 < n < 0.85$, this indicates anomalous (non-Fickian) transport, involving simultaneous Fickian molecular diffusion of curcumin through the polymer matrix and polymer chain relaxation/erosion of the PLGA backbone. This dual mechanism is consistent with PLGA's known combined diffusion-erosion release behavior and explains the extended sustained release profile observed over 72 hours.

Table 7: Drug Release Kinetics of F2 Nanoparticles.

Kinetic Model	R ² Value	Interpretation
Zero Order	0.942	Concentration-independent release
First Order	0.968	Release proportional to remaining drug
Higuchi	0.981	Diffusion from matrix
Korsmeyer-Peppas (n=0.68)	0.995 (BEST)	Anomalous non-Fickian transport

5.8 Capsule Evaluation

Hard gelatin capsules filled with optimized F2 nanoparticle lyophilizate were evaluated against IP 2018 pharmacopoeial specifications. All parameters demonstrated compliance (Table 8). Rapid disintegration (8.4 ± 1.2 min, well within the 15-min IP limit) releases the nanoparticle powder efficiently into gastrointestinal fluid. Dissolution of $74.2 \pm 3.1\%$ drug at 45 min exceeds the pharmacopoeial Q value of 70%, confirming adequate drug availability from the finished dosage form. Drug content of $96.8 \pm 2.1\%$ is well within the 85–115% specification, demonstrating formulation reproducibility and content uniformity.

Table 8: Capsule Evaluation Results (F2 Optimized Batch).

Parameter	Result	IP 2018 Specification	Compliance
Appearance	Orange capsules, smooth, defect-free	Elegance & uniformity	PASS
Average Fill Weight	648 \pm 18 mg	—	—
Weight Variation (max dev.)	$\pm 6.2\%$	$\pm 7.5\%$	PASS
Drug Content	96.8 \pm 2.1%	85–115% of label	PASS
Disintegration Time	8.4 \pm 1.2 min	≤ 15 min	PASS
Dissolution at 45 min	74.2 \pm 3.1%	$\geq 70\%$ (Q value)	PASS
Carr's Index (powder)	13.2%	$< 16\%$ (Good flow)	PASS
Angle of Repose	27.4°	$< 30^\circ$ (Excellent)	PASS

6. CONCLUSION

This study presents a comprehensive, validated pharmaceutical workflow — from *Curcuma longa* rhizome processing to a finished pharmacopoeially compliant oral dosage form — demonstrating that PLGA nanoparticle encapsulation is an effective, reproducible, and scalable strategy to address curcumin's critical biopharmaceutical limitations. Curcumin was successfully extracted with a mean yield of $5.86 \pm 0.31\%$ w/w by optimized Soxhlet extraction (95% ethanol, 60°C, 6 h), demonstrating excellent batch-to-batch reproducibility (RSD 5.3%). Isolated curcumin was confirmed pure by sharp melting point (182–183°C), single-spot TLC (Rf 0.52), concordant paper chromatography (Rf 0.70), and UV-Vis spectral overlay with reference standard (λ -max 427 nm, $R^2 = 0.9994$). Optimized PLGA nanoparticles (F2; curcumin:PLGA 1:10; PVA 1.0%) achieved particle size 218 nm, PDI 0.22, zeta potential -24.8 mV, EE% 78.6%, and DL% 7.4%, meeting all pre-set acceptance criteria.

In vitro drug release followed Korsmeyer-Peppas kinetics ($n = 0.68$, $R^2 = 0.995$) with biphasic profile: initial burst (22% at 2 h) followed by sustained release (83% at 72 h) — superior to plain curcumin suspension. Hard gelatin capsules of F2 nanoparticles complied with all IP 2018 pharmacopoeial specifications, confirming pharmaceutical acceptability of the finished dosage form.

This work establishes curcumin-PLGA nanoparticles as a rational, evidence-based platform formulation for oral delivery, with potential applications across anti-inflammatory, oncological, metabolic, and neurodegenerative disease areas. Future directions include in vivo pharmacokinetic studies in Wistar rat models, pharmacodynamic evaluation in disease-specific animal models, ICH Q1A stability studies for shelf-life determination, and regulatory scale-up towards clinical translation.

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