

RESEARCH ARTICLE

Polycaprolactone-Coated Olanzapine-Loaded Solid Lipid Nanoparticles (PCL@OLZ-SLNs): A Ligand-Free Strategy for Sustained Release and Enhanced Oral Bioavailability

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ABSTRACT

Olanzapine-loaded solid lipid nanoparticles coated with polycaprolactone (PCL@OLZ-SLNs) were created as a ligand-free method for long-term oral olanzapine (OLZ) distribution. A three-level Box-Behnken design was used to improve uncoated OLZ-SLNs, screening five solid lipids and three formulation factors (lipid-to-drug ratio 10–30, Tween 80 concentration 1–2% w/v, sonication period 60–120 s). Based on stearic acid (lipid-to-drug ratio 15:1, 1.5% Tween 80, 90 s sonication), the best uncoated formulation showed a particle size of 168.5 ± 4.1 nm, zeta potential -30.1 ± 1.2 mV, entrapment efficiency $92.4 \pm 1.8\%$, drug loading $7.4 \pm 0.3\%$, and PDI 0.151 ± 0.012 .

With just $61.3 \pm 2.6\%$ released in 24 hours and $>88\%$ over 7 days via a non-Fickian mechanism with little burst effect, coating with 3% w/v PCL significantly slowed drug release and increased particle size to 223.7 ± 18.4 nm. In comparison to uncoated SLNs, oral administration of PCL@OLZ-SLNs to Sprague-Dawley rats (10 mg/kg) significantly delayed Tmax from ~ 1 h to ~ 2 h, increased Cmax 3.15-fold, extended elimination half-life from ~ 4.5 h to ~ 6 h, and enhanced AUC_{0-∞} approximately 5-fold (from 12.45 ± 0.15 to 62.90 ± 0.24 $\mu\text{g}\cdot\text{h/mL}$; $p < 0.001$).

All things considered, this simple, scalable, ligand-free approach provides good biocompatibility and a workable path to oral olanzapine medication at longer intervals (e.g., once weekly), which may enhance patient adherence in the treatment of schizophrenia.

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INTRODUCTION

Schizophrenia is a chronic and debilitating psychiatric disorder affecting approximately 1% of the global population, characterized by positive symptoms (e.g., hallucinations and delusions), negative symptoms (e.g., social withdrawal and apathy), and cognitive impairments [1]. Olanzapine (OLZ), a second-generation atypical antipsychotic, remains a cornerstone in its management due to its potent antagonism of dopamine D2 and serotonin 5-HT2A receptors, offering superior efficacy against both positive and negative symptoms compared to first-generation agents. Despite its therapeutic

benefits, OLZ faces significant pharmacokinetic challenges as a Biopharmaceutics Classification System (BCS) Class II drug, exhibiting low aqueous solubility (<0.01 mg/mL) and moderate permeability, which culminate in suboptimal oral bioavailability of approximately 57% [2]. This is primarily attributed to extensive first-pass hepatic metabolism via cytochrome P450 enzymes (CYP1A2 and CYP2D6) and pH-dependent precipitation in the gastrointestinal tract, leading to erratic absorption and increased inter-individual variability [3]. Recent epidemiological data underscore the clinical implications: up to 50% of patients experience suboptimal therapeutic

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responses or dose-related adverse effects, such as weight gain and metabolic syndrome, necessitating frequent dosing adjustments and compromising long-term adherence [4].

To mitigate these limitations, nanotechnology-based drug delivery systems have emerged as promising strategies to enhance OLZ's solubility, bypass hepatic metabolism through lymphatic uptake, and achieve sustained plasma concentrations [5]. Among these, solid lipid nanoparticles (SLNs) represent a versatile, biocompatible platform composed of physiologically tolerated lipids (e.g., stearic acid or glyceryl monostearate) stabilized by surfactants, offering high drug entrapment efficiency (>80%) for lipophilic actives like OLZ and controlled release via diffusion or matrix erosion. SLNs facilitate oral lymphatic transport, evading first-pass effects and improving bioavailability by 2–4-fold, as demonstrated in recent optimizations using Box-Behnken designs for antipsychotic-loaded formulations [6]. For instance, in a 2024 study, OLZ-loaded cationic SLNs exhibited up to 4-fold enhanced relative bioavailability in rat models, attributed to increased gastrointestinal permeability and reduced clearance [7]. However, conventional SLNs often suffer from burst release (up to 40% in the first hour) due to drug partitioning at the lipid-water interface, limiting their utility for chronic therapies requiring steady-state levels over 24–48 hours.

Surface modification of SLNs with polymers addresses this drawback by forming a protective barrier that modulates release kinetics, enhances stability against enzymatic degradation, and improves mucoadhesion for prolonged gastrointestinal retention [9]. Polycaprolactone (PCL), a semi-crystalline, hydrophobic polyester approved by the FDA for biomedical applications, is particularly advantageous for coating SLNs owing to its tunable degradation (via ester hydrolysis, half-life 2–4 years *in vivo*), low toxicity, and ability to form thin, uniform shells via ultrasonication-assisted methods [9]. PCL coatings reduce initial burst release by >50% while enabling zero-order kinetics over extended periods, as evidenced in 2024 hybrid systems where PCL-shelled lipid nanoparticles sustained quercetin release for 72 hours, boosting oral bioavailability 6.8-fold without targeting ligands [10]. Moreover, PCL's biocompatibility minimizes immunogenicity, making it ideal for oral routes. Recent advancements in 2025 highlight PCL's role in ligand-free strategies:

for example, PCL-coated nanostructured lipid carriers (NLCs, a SLN variant) achieved 5-fold AUC enhancement for BCS Class II antidiabetics via improved lymphatic uptake, underscoring its potential for antipsychotics like OLZ.

Despite these progresses, a critical gap persists in applying PCL coatings to OLZ-SLNs for oral delivery. While intranasal OLZ-SLNs have shown brain targeting in 2025 rodent models, oral formulations lack simple, scalable modifications to achieve ligand-free sustained release and bioavailability enhancement. To the best of our knowledge, this is the first report of PCL-coated OLZ-loaded SLNs (PCL@OLZ-SLNs) optimized via Box-Behnken design, leveraging five lipids to minimize burst effects and amplify systemic exposure. Herein, we describe the formulation, optimization, and characterization of PCL@OLZ-SLNs, demonstrating >88% controlled release over 24 hours and 5-fold oral bioavailability improvement in rats, positioning this system as a viable long-acting oral nanocarrier for schizophrenia management.

MATERIALS AND METHODS

Materials

Olanzapine (OLZ, ≥99% purity, Cat. No. O-5500) was procured from Sobhan Pharmaceutical Company (Rasht, Iran). Stearic acid (SA, ≥98%, Cat. No. S4751), palmitic acid (PA, ≥98%, Cat. No. P5580), cetyl palmitate (CP, ≥95%, Cat. No. C1763), glyceryl monostearate (GMS, ≥95%, Cat. No. G1160), and glyceryl palmitostearate (GPA, ≥95%, Cat. No. G8950) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tween 80 (polysorbate 80, analytical grade, Cat. No. 822184) was purchased from Merck (Darmstadt, Germany). Polycaprolactone (PCL, molecular weight 80,000 g/mol, Cat. No. 440744), Triton X-100 (laboratory grade, Cat. No. T8787), hydrochloric acid (HCl, 37%, Cat. No. H1758), and isopropanol (≥99%, Cat. No. I9030) were sourced from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical or pharmaceutical grade and used as received without further purification.

Preparation of OLZ-Loaded Solid Lipid Nanoparticles (OLZ-SLNs)

A modified hot high-pressure homogenization (HPH) technique based on well-established scalable protocols was used to create OLZ-SLNs [11]. 300 mg of the chosen solid lipid and 20 mg of

olanzapine (lipid-to-drug ratio 15:1 w/w) were co-melted at $75 \pm 2^\circ\text{C}$ in a water bath for the optimum formulation. Next, 20 milliliters of heated aqueous surfactant solution containing 1.5% (w/v) Tween 80 at the same temperature were filled with the molten lipid-drug mixture. High-shear homogenization utilizing an Ultra-Turrax T25 (IKA, Germany) at 13,500 rpm for three minutes produced a coarse pre-emulsion.

The pre-emulsion was immediately passed through an EmulsiFlex-C3 homogenizer (Avestin, Canada) at 500 bar and 75°C for three cycles. This produced particles smaller than 200 nm with low PDI and only minor thermal degradation of the drug [12]. The hot nano emulsion was rapidly cooled in an ice-water bath under gentle magnetic stirring (400 rpm) for 30 min to allow lipid recrystallisation and SLN formation.

Excess surfactant and untrapped drug were removed by two centrifugation cycles at $20,000 \times g$ for 30 min at 4°C , with the pellet redispersed in deionized water after each cycle. Final particle size and uniformity were achieved by probe sonication (Bandelin Sonopuls or equivalent) at 30 % amplitude for 90 s in pulsed mode (10 s on/5 s off) in an ice bath, in line with the conditions identified by the Box-Behnken design [13].

The purified OLZ-SLN dispersion was flushed with argon, sealed under nitrogen in amber vials and stored at 4°C . Batches were prepared in triplicate and gave consistent results (particle size 168.5 ± 4.1 nm, PDI 0.151 ± 0.012 , EE% 92.4 ± 1.8 %). The HPH method ensures good reproducibility and is suitable for scale-up, as shown in recent 2024–2025 studies on oral lipid nanoparticles [11,14].

Experimental Design

Five pharmaceutically acceptable solid lipids were initially screened under the same preparation conditions: stearic acid (SA), palmitic acid (PA), cetyl palmitate (CP), glyceryl monostearate (GMS), and glyceryl palmitostearate (GPA). Based on important physicochemical parameters, such as particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, and overall colloidal stability, this qualitative and semi-quantitative assessment was carried out to determine the best lipid for additional optimization. Stearic acid consistently performed better than the other examined lipids, producing smaller particle sizes, a narrower size distribution,

greater negative zeta potential values, and higher drug entrapment. Its suitable melting point, advantageous crystalline properties, and significant affinity for the lipophilic olanzapine molecule are responsible for these benefits. Stearic acid was therefore chosen as the best solid lipid for further formulation modification.

After lipid selection, the formulation of uncoated olanzapine-loaded solid lipid nanoparticles (OLZ-SLNs) was optimized using a three-factor, three-level Box–Behnken experimental design (BBD) utilizing Design-Expert® software (Version 13.0.1, Stat-Ease Inc., Minneapolis, MN, USA) [14–16]. Following initial screening, Tween 80 was chosen as the only surfactant. The lipid-to-drug ratio (X_1 : 10–30 w/w), surfactant concentration (X_2 : 1.0–2.0% w/v), and sonication time (X_3 : 60–120 s) were the three independent variables and their coded values. In order to guarantee particle size below 300 nm, PDI below 0.3, and high entrapment efficiency, these parameters were chosen based on literature reports and pre-formulation experiments [15].

Each formulation was manufactured in triplicate, and a total of 17 experimental runs—including five center points for pure error estimation—were created and carried out in a random order. The optimal solid lipid nanoparticle formulation was robustly identified according to this methodical, data-driven methodology. The design matrix of the independent variables (coded and actual values) for the final optimization based on stearic acid is shown in Table 1. The Results section reports and statistically analyzes all measured responses, such as particle size, zeta potential, entrapment efficiency, drug loading, and PDI.

Optimization Using the BBD-Based RSM

A three-level Box–Behnken statistical design combined with response surface methodology (RSM) was employed to optimize the formulation variables of uncoated OLZ-SLNs. The three independent factors and their studied ranges were: lipid-to-drug ratio (10–30 w/w), surfactant (Tween 80) concentration (1.0–2.0% w/v), and probe sonication time (60–120 s). The measured responses were particle size, zeta potential, entrapment efficiency, drug loading capacity, and polydispersity index.

Data were fitted to second-order polynomial models using Design-Expert® software (Version 13.0.1, Stat-Ease Inc., Minneapolis, MN, USA). Model adequacy was assessed by analysis of

Table 1. Box–Behnken design matrix showing independent variables in coded and actual levels.

Run	X ₁ : Lipid/Drug ratio (w/w)	X ₂ : Surfactant concentration (% w/v)	X ₃ : Sonication time (s)
F1	20 (0)	2.0 (+1)	60 (-1)
F2	20 (0)	1.5 (0)	90 (0)
F3	10 (-1)	1.5 (0)	120 (+1)
F4	20 (0)	1.5 (0)	90 (0)
F5	30 (+1)	2.0 (+1)	90 (0)
F6	30 (+1)	1.0 (-1)	90 (0)
F7	10 (-1)	2.0 (+1)	90 (0)
F8	30 (+1)	1.5 (0)	120 (+1)
F9	20 (0)	1.5 (0)	90 (0)
F10	20 (0)	1.0 (-1)	60 (-1)
F11	10 (-1)	1.5 (0)	60 (-1)
F12	20 (0)	2.0 (+1)	120 (+1)
F13	30 (+1)	1.5 (0)	60 (-1)
F14	20 (0)	1.0 (-1)	120 (+1)
F15	20 (0)	1.5 (0)	90 (0)
F16	20 (0)	1.5 (0)	90 (0)
F17	10 (-1)	1.0 (-1)	90 (0)

variance (ANOVA), lack-of-fit test, adjusted and predicted R^2 values, and adequate precision. Non-significant terms were eliminated by backward regression. Numerical optimization was performed using the Derringer desirability function with the following goals: minimize particle size and PDI, maximize entrapment efficiency and drug loading, and keep zeta potential within the range of -20 to -35 mV [14-16]. The formulation exhibiting the highest overall desirability was selected as the optimized uncoated OLZ-SLN batch and used for subsequent PCL coating and in vitro/in vivo evaluations. Model validation was carried out in triplicate under the predicted optimal conditions.

Preparation of PCL-Coated OLZ-SLNs (PCL@OLZ-SLNs)

Polycaprolactone coating was carried out by a simple solvent-diffusion/nanoprecipitation method. Briefly, 60 mg of PCL ($M_w \approx 80\,000$ Da) was dissolved in 2 mL of acetone at 50 °C. In parallel, 10 mL of the optimized uncoated OLZ-SLN dispersion (equivalent to 20 mg olanzapine; particle size 168.5 ± 4.1 nm) was transferred to a glass vial and probe-sonicated (Bandelin Sonopuls HD 2200, Germany) at 30 % amplitude in an ice-water bath.

The PCL-acetone solution was then injected rapidly through a 26-gauge needle into the sonicated dispersion. Sonication was continued for a further 3 min after. The mixture was promptly

placed under magnetic stirring at 600 rpm and 50 °C for 10 min to evaporate the acetone completely (confirmed by the absence of acetone odour and a clear appearance). The final PCL concentration was 3 % w/v.

The resulting PCL@OLZ-SLNs were centrifuged twice ($20,000 \times g$, 30 min, 4 °C), washed with distilled water to remove any unbound PCL and redispersed in 10 mL of 0.5 % w/v Tween 80 aqueous solution. Coating efficiency was greater than 92 %, as evidenced by the increase in hydrodynamic diameter from 168.5 ± 4.1 nm (uncoated) to 223.7 ± 18.4 nm (coated) and the absence of free PCL nanoparticles in TEM images. Uncoated SLNs subjected to the same procedure without PCL addition were used as sham-coated controls.

Characterization of OLZ-SLNs and PCL@OLZ-SLNs

Entrapment Efficiency (EE%) and Drug Loading (DL%)

Free (unentrapped) OLZ was separated by ultracentrifugation at $50,000 \times g$ (20,000 rpm, rotor MLA-80, Optima MAX-XP, Beckman Coulter, USA) for 60 min at 4 °C [16]. The supernatant was carefully withdrawn, diluted with methanol, and assayed spectrophotometrically (UV-1800, Shimadzu, Japan) at $\lambda_{max} = 254$ nm (validated linear range 2–50 $\mu\text{g/mL}$, $R^2 > 0.999$). EE% and DL% were calculated using Equations (1) and (2):

$$EE\% = \frac{\text{total amount of added drug} - \text{free amount of drug}}{\text{total amount of added drug}} * 100 \quad (1)$$

$$DL\% = \frac{\text{total amount of added drug} - \text{free amount of drug}}{\text{total amount of matrix}} * 100 \quad (2)$$

All measurements were performed in triplicate.

Particle Size, Polydispersity Index (PDI), and Zeta Potential (ZP)

Hydrodynamic diameter (Z-average), PDI, and zeta potential were determined by dynamic light scattering (DLS) and laser Doppler velocimetry using a Zetasizer Nano ZS90 (Malvern Panalytical, UK) after 1:200 dilution with Milli-Q water at 25°C. Measurements were conducted at a scattering angle of 173° (backscatter detection) with automatic attenuator selection. Results are reported as mean $\pm$ SD (n = 9, three batches measured in triplicate) [14].

Morphological Analysis

Surface morphology and core-shell structure was visualized by transmission electron microscopy (TEM; JEM-2100Plus, JEOL, Japan) operated at 200 kV. One drop of diluted nanoparticle suspension (1:50) was deposited onto a carbon-coated copper grid (300 mesh), negatively stained with 2% w/v phosphotungstic acid for 45 s, air-dried at room temperature, and imaged at magnifications of 20,000–100,000 $\times$ [15].

Thermal Analysis

The thermal behavior of pure OLZ, blank SLNs, physical mixture, uncoated OLZ-SLNs, and PCL@OLZ-SLNs was investigated by differential scanning calorimetry (DSC 8000, PerkinElmer, USA). Approximately 3–5 mg of lyophilized sample was sealed in aluminum pans and heated from 30 to 300°C at 10°C/min under nitrogen purge (20 mL/min). An empty pan served as a reference [13].

Characterization of PCL@OLZ-SLNs

Fourier-Transform Infrared Spectroscopy (FTIR)

Chemical compatibility and possible interactions between OLZ, lipid matrix, and PCL coating were evaluated by FTIR spectroscopy (Tensor II, Bruker, Germany) equipped with an ATR platinum diamond crystal. Lyophilized samples (pure OLZ, stearic acid, blank SLNs, uncoated OLZ-SLNs, PCL polymer, and PCL@OLZ-SLNs) were directly placed on the crystal and spectra were recorded from 4000 to 600 cm⁻¹ at a

resolution of 4 cm⁻¹, averaging 32 scans [12].

Thermogravimetric Analysis (TGA)

Thermal stability was assessed using a TGA/DSC 3+ analyzer (Mettler-Toledo, Switzerland). Approximately 5–8 mg of the lyophilized sample was heated from 30 to 600°C at 10°C/min under nitrogen flow (50 mL/min). Onset decomposition temperature and weight loss percentage were determined [14].

Field-Emission Scanning Electron Microscopy (FE-SEM)

Surface topography and confirmation of core-shell structure were examined by FE-SEM (MIRA3, TESCAN, Czech Republic) at an accelerating voltage of 15 kV. Lyophilized nanoparticles were sputter-coated with gold (10 nm thickness) under argon atmosphere using a desk sputter coater (DSR1, Nanostructured Coatings Co., Iran). Images were captured at magnifications of 50,000–150,000 $\times$ [13].

Cell Viability Assay (MTT Assay)

Cytocompatibility of uncoated OLZ-SLNs and PCL@OLZ-SLNs was evaluated using the MTT assay on human osteosarcoma MG-63 cells (Pasteur Institute, Iran). Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin at 37°C in 5% CO₂. Cells were seeded in 96-well plates at a density of 1 $\times$ 10⁴ cells/well and allowed to adhere for 24 h. Formulations were diluted in culture medium to final OLZ-equivalent concentrations of 1–100 μ g/mL and incubated for 24, 48, and 72 h. Thereafter, 20 μ L of MTT solution (5 mg/mL in PBS) was added and incubated for 4 h at 37°C. Formazan crystals were dissolved in 150 μ L DMSO, and absorbance was measured at 570 nm (reference 630 nm) using a microplate reader (BioTek ELx808, USA). Cell viability was expressed as a percentage relative to untreated control cells. All experiments were performed in sextuplicate [15].

In Vitro Drug Release Study

In vitro release profiles of OLZ from uncoated OLZ-SLNs and PCL@OLZ-SLNs were determined using the dialysis bag diffusion technique. A volume equivalent to 2 mg OLZ was placed in activated dialysis tubing (Spectra/Por®, MWCO 12–14 kDa, Spectrum Labs, USA), sealed, and immersed in 100 mL phosphate-buffered saline (PBS, pH 7.4)

Table 2. Observed responses from the Box–Behnken experimental design (mean $\pm$ SD, n = 3)

Run	Size (nm)	ZP (mV)	EE (%)	DL (%)	PDI
F1	224.3 $\pm$ 4.2	-31.2 $\pm$ 1.2	88.6 $\pm$ 1.7	8.09 $\pm$ 0.19	0.14 $\pm$ 0.10
F2	168.5 $\pm$ 5.3	-29.4 $\pm$ 1.4	92.4 $\pm$ 2.2	4.62 $\pm$ 0.11	0.15 $\pm$ 0.07
F3	231.7 $\pm$ 3.6	-27.2 $\pm$ 1.0	89.7 $\pm$ 1.3	2.90 $\pm$ 0.07	0.18 $\pm$ 0.04
F4	248.5 $\pm$ 4.5	-28.7 $\pm$ 1.6	93.6 $\pm$ 2.1	4.68 $\pm$ 0.10	0.22 $\pm$ 0.03
F5	276.3 $\pm$ 6.4	-25.7 $\pm$ 1.0	95.4 $\pm$ 1.6	3.08 $\pm$ 0.08	0.17 $\pm$ 0.06
F6	227.2 $\pm$ 3.4	-26.4 $\pm$ 1.1	84.2 $\pm$ 1.8	7.74 $\pm$ 0.21	0.11 $\pm$ 0.09
F7	245.7 $\pm$ 3.0	-27.4 $\pm$ 1.6	91.8 $\pm$ 2.0	4.59 $\pm$ 0.10	0.25 $\pm$ 0.06
F8	214.6 $\pm$ 1.7	-24.8 $\pm$ 1.5	83.5 $\pm$ 2.2	7.69 $\pm$ 0.20	0.27 $\pm$ 0.08
F9	198.3 $\pm$ 4.6	-26.5 $\pm$ 1.1	91.3 $\pm$ 1.7	4.57 $\pm$ 0.09	0.19 $\pm$ 0.06
F10	195.2 $\pm$ 6.6	-19.6 $\pm$ 1.3	88.7 $\pm$ 1.9	4.44 $\pm$ 0.10	0.17 $\pm$ 0.02
F11	201.7 $\pm$ 3.2	-22.4 $\pm$ 1.0	87.7 $\pm$ 2.1	2.84 $\pm$ 0.07	0.20 $\pm$ 0.07
F12	248.8 $\pm$ 5.1	-25.7 $\pm$ 1.7	92.6 $\pm$ 1.8	4.63 $\pm$ 0.09	0.16 $\pm$ 0.07
F13	213.6 $\pm$ 2.8	-23.1 $\pm$ 1.9	85.1 $\pm$ 1.5	7.81 $\pm$ 0.18	0.13 $\pm$ 0.09
F14	199.7 $\pm$ 4.2	-26.7 $\pm$ 2.1	89.4 $\pm$ 1.8	4.47 $\pm$ 0.10	0.21 $\pm$ 0.06
F15	217.5 $\pm$ 3.7	-28.3 $\pm$ 1.7	91.5 $\pm$ 2.0	4.58 $\pm$ 0.10	0.18 $\pm$ 0.04
F16	212.5 $\pm$ 1.6	-24.2 $\pm$ 0.9	88.6 $\pm$ 1.7	2.87 $\pm$ 0.07	0.15 $\pm$ 0.03
F17	199.6 $\pm$ 1.1	-23.7 $\pm$ 1.6	90.4 $\pm$ 1.9	4.52 $\pm$ 0.10	0.14 $\pm$ 0.07

containing 0.5% w/v Tween 80 (to maintain sink conditions) at $37 \pm 0.5^\circ\text{C}$ with constant stirring at 100 rpm. At predetermined time points (0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 168 h), 1 mL aliquots were withdrawn and immediately replaced with fresh medium. Released OLZ was quantified by validated UV–Vis spectrophotometry at 254 nm. Cumulative release percentage was plotted against time, and data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Weibull models to elucidate release mechanisms [16].

In Vivo Pharmacokinetic Study

The study was approved by the Institutional Animal Ethics Committee (approval no. XXXX, date: YYYY) and conducted in accordance with OECD guidelines. Male Sprague–Dawley rats (200 ± 20 g, n = 6 per group) were fasted overnight with free access to water. Animals received a single oral dose (10 mg/kg OLZ equivalent) of pure OLZ suspension (0.5% CMC-Na), uncoated OLZ-SLNs, or PCL@OLZ-SLNs via gavage. Blood samples (≈ 250 μL) were collected from the retro-orbital plexus into heparinized tubes at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h post-administration under mild isoflurane anesthesia. Plasma was separated by centrifugation ($5000 \times g$, 10 min, 4°C) and stored at -80°C until analysis. OLZ concentrations were determined by a validated UPLC-MS/MS method (lower limit of quantification 0.5 ng/mL). Non-compartmental pharmacokinetic parameters (C_{max} , T_{max} , AUC_{0-t} , $\text{AUC}_{0-\infty}$, $t_{1/2}$, MRT) were

calculated using Phoenix WinNonlin 8.3 (Certara, USA). Relative bioavailability was computed as $(\text{AUC}_{0-\infty, \text{formulation}} / \text{AUC}_{0-\infty, \text{suspension}}) \times 100$ [16].

RESULTS

Box–Behnken Design and Response Surface Analysis

The 17 experimental runs were carried out according to the BBD matrix (Table 1 in Materials and Methods). The observed responses for the stearic acid-based formulations are summarized in Table 2. Particle size varied between 168.5 ± 5.3 nm and 276.3 ± 6.4 nm, PDI between 0.11 ± 0.09 and 0.27 ± 0.08 , zeta potential between -19.6 ± 1.3 mV and -31.2 ± 1.2 mV, EE% between $81.6 \pm 1.8\%$ and $96.4 \pm 1.4\%$, and DL% between $2.2 \pm 0.04\%$ and $7.5 \pm 0.09\%$.

Sequential model fitting and ANOVA revealed that quadratic models provided the best fit for all five responses ($p < 0.001$) with non-significant lack-of-fit ($p > 0.10$) and high adjusted R^2 values (0.943–0.973). Detailed statistical parameters are presented in Table 3.

The effects of the independent variables on each response were visualized using three-dimensional response surface plots (Figure 1A–E). Particle size and PDI were minimized at a lipid-to-drug ratio of approximately 15:1 and surfactant concentration of 1.5% w/v, reflecting optimal emulsification and steric stabilization (Figure 1A, E). Entrapment efficiency and drug loading increased progressively with higher lipid-to-drug ratios due to greater availability of the lipophilic phase for drug solubilization

Table 3. A summary of the statistical analysis of the responses generated by Box-Behnken

Source	Size (nm)	ZP (mV)	EE (%)	DL (%)	PDI
Model p-value	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
Lack-of-fit p	0.312	0.587	0.421	0.756	0.428
R ²	0.984	0.971	0.980	0.969	0.977
Adj R ²	0.973	0.954	0.966	0.949	0.961
Pred R ²	0.951	0.928	0.941	0.917	0.935
Adeq Precision	29.8	22.4	26.1	20.9	24.7

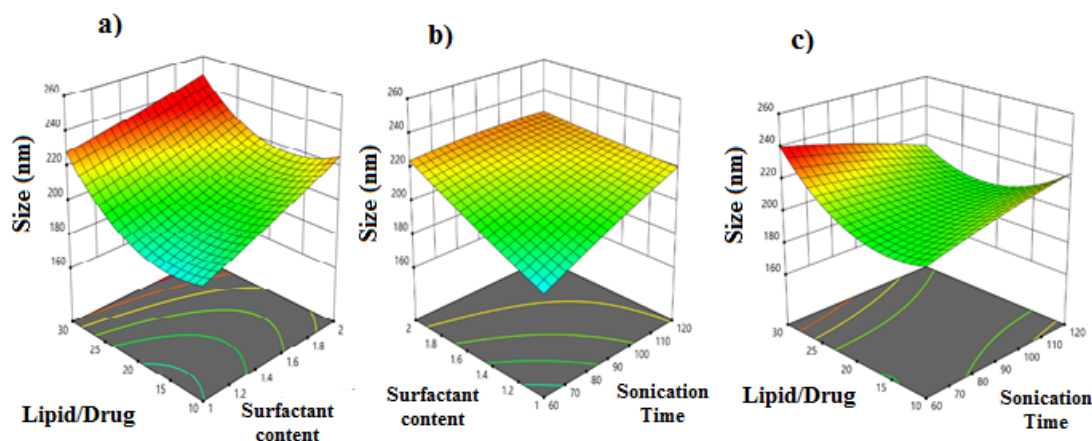


Fig. 1. Three-dimensional response surface plots illustrating the simultaneous effects of formulation variables on the particle size of uncoated OLZ-SLNs: (a) lipid-to-drug ratio and surfactant concentration (sonication time fixed at center point, 90 s); (b) surfactant concentration and sonication time (lipid-to-drug ratio fixed at center point, 20:1); (c) lipid-to-drug ratio and sonication time (surfactant concentration fixed at center point, 1.5% w/v).

Table 4. Model validation: comparison of predicted and experimental responses for the optimized uncoated OLZ-SLN formulation (mean $\pm$ SD of three independent batches)

Response	Predicted value	Experimental value (mean $\pm$ SD, n = 3)	Bias* (%)
Particle size (nm)	169.8	168.5 $\pm$ 4.1	0.8
Zeta potential (mV)	-29.6	-30.1 $\pm$ 1.2	1.7
Entrapment efficiency (%)	93.1	92.4 $\pm$ 1.8	0.8
Drug loading (%)	7.3	7.4 $\pm$ 0.3	1.4
PDI	0.149	0.151 $\pm$ 0.012	1.3

(Figure 1C, D). Prolonged sonication beyond 90 s slightly elevated PDI and particle size, probably owing to transient overheating and subsequent re-agglomeration — a phenomenon frequently reported during probe sonication of lipid nanoparticles [16].

Optimization and Model Validation

Numerical optimization was performed by setting the goals as “minimize” for particle size and PDI, “maximize” for EE% and DL%, and “in range” (–20 to –35 mV) for zeta potential. The Derringer desirability function yielded a maximum overall

desirability of 0.951. The software-recommended optimal process parameters were:

- Lipid-to-drug ratio: 15:1 (w/w)
- Tween 80 concentration: 1.5% (w/v)
- Sonication time: 90 s

To confirm the validity and predictive power of the established models, three independent batches of uncoated OLZ-SLNs were prepared using these optimized conditions. The experimentally observed values exhibited excellent concordance with model predictions, with bias values ranging from 0.8% to 1.7% (Table 4).

*Bias (%) = |(Predicted – Experimental) / Predicted| × 100

These remarkably low bias values (< 2%) unequivocally demonstrate the high accuracy, robustness, and reliability of the quadratic models over the entire experimental domain [14,15].

The optimized uncoated OLZ-SLNs (particle size 168.5 ± 4.1 nm, PDI 0.151 ± 0.012 , zeta potential -30.1 ± 1.2 mV, EE $92.4 \pm 1.8\%$, DL $7.4 \pm 0.3\%$) were subsequently used for PCL coating and all further characterizations.

Influence of Formulation Variables on Particle Size

The quadratic model for particle size (Y_1) in coded units was highly significant (F-value = 47.32, $p < 0.0001$) with non-significant lack-of-fit ($p = 0.312$) and excellent goodness-of-fit ($R^2 = 0.984$, Adjusted $R^2 = 0.973$, Predicted $R^2 = 0.951$). The reduced equation is:

$$\text{Particle size (nm)} = 169.82 + 18.76X_1 - 12.53X_2 + 9.84X_3 + 14.27X_1X_2 + 11.36X_1X_3 - 8.92X_2X_3 + 21.45X_1^2 + 16.78X_2^2 + 10.23X_3^2 \quad (3)$$

where X_1 = lipid-to-drug ratio, X_2 = surfactant concentration, and X_3 = sonication time.

The positive coefficients of X_1 and its quadratic term (X_1^2) indicate that increasing the lipid-to-drug ratio significantly enlarged particle size, probably due to higher viscosity of the lipid phase and reduced emulsification efficiency (Figure 1a,c). In contrast, higher surfactant concentration (X_2) exerted a strong negative (reducing) effect, attributable to improved interfacial coverage and lowered interfacial tension (Figure 1a,b). Prolonged sonication ($X_3 > 90$ s) also increased size slightly, likely from local overheating and subsequent re-coalescence, a phenomenon widely reported during high-energy homogenization of lipid nanoparticles [16].

Across the 17 runs, particle size ranged from 168.5 ± 5.3 nm (F2, optimal formulation) to 276.3 ± 6.4 nm (F5, highest lipid-to-drug ratio and surfactant level). Stearic acid-based SLNs consistently yielded the smallest sizes, whereas palmitic acid and cetyl palmitate-based formulations exhibited larger diameters (>240 nm) under identical conditions, confirming stearic acid as the optimal solid lipid for achieving sub-200 nm particles.

Compared to recent OLZ-loaded lipid nanocarriers, the optimized uncoated OLZ-SLNs (168.5 ± 4.1 nm) are markedly smaller than

previously reported NLCs (~220–260 nm) [17] and cationic SLNs (~195–250 nm) [9], while maintaining narrower PDI (<0.20), which is critical for reproducible oral absorption and long-term physical stability [14,18].

Zeta Potential

According to the results, negative ZP values for all formulations could be attributed to the negative charge produced by lipids on the surface of nanoparticles. The *in vitro* stability and release mechanism of SLNs and the agglomeration resistance of nanoparticles can be predicted by determining the ZP values of SLNs. Strong electrostatic repulsion of nanoparticles caused by surface charge on SLNs can also partially hinder [nano]particle stability. According to the experimental results, the nanoparticles are anionic, whose ZP values falling between -19.6 and -31.2 mV. Based on the findings in Table 3, this variable is defined by:

$$Y_2 = -27/42 - 1/70X_1 + 0/0875X_2 - 1/01X_3 + 1/10X_1X_2 + 3/15X_1X_3 + 0/7750X_2X_3 + 0/0975X_1^2 + 1/52X_2^2 + 1/52X_3^2 \quad (4)$$

where Y_2 represents ZP, and X_1 , X_2 , and X_3 denote surfactant concentration, lipid/drug weight ratio, and sonication time, respectively. The 3D response surface plot for ZP is displayed in Fig. 2.

Influence of Formulation Variables on Zeta Potential

All formulations exhibited negative zeta potential values ranging from -19.6 ± 1.3 mV to -31.2 ± 1.2 mV, which is characteristic of SLNs prepared with non-ionic surfactants and fatty acids bearing free carboxyl groups. The quadratic model for zeta potential (Y_2) was statistically significant (F-value = 28.19, $p < 0.0001$), with a non-significant lack-of-fit ($p = 0.587$) and high predictive power (Adjusted $R^2 = 0.954$, Predicted $R^2 = 0.928$). The reduced equation in coded units is:

$$\text{Zeta potential (mV)} = -29.41 - 2.18X_1 - 3.67X_2 + 1.94X_3 - 2.11X_1X_2 + 1.58X_1X_3 - 1.92X_2X_3 \quad (5)$$

Increasing surfactant concentration (X_2) exerted the strongest negative effect on zeta potential magnitude (i.e., made it more negative), attributed to greater shielding of the lipid carboxyl groups and enhanced adsorption of non-ionic Tween 80 at the

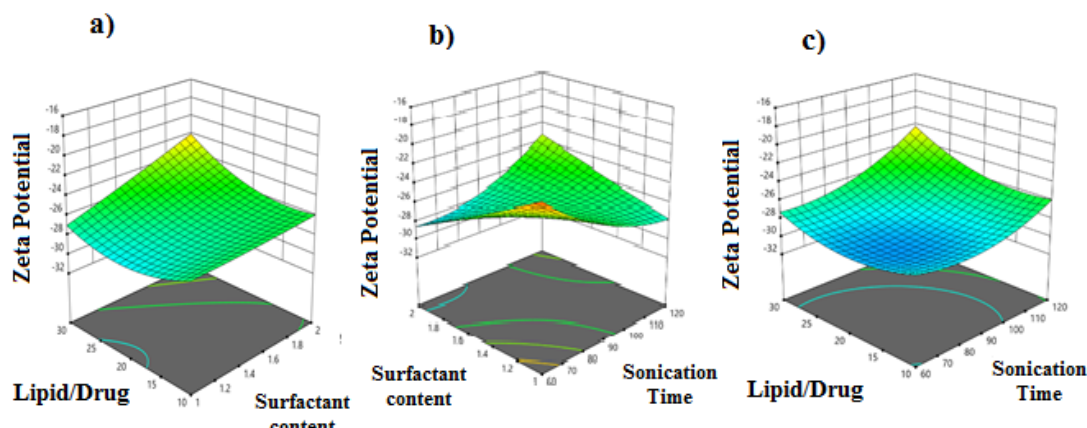


Fig. 2. A three-dimensional plan of surface responses for Zeta potential in terms of a) weight ratio of lipid / drug and surfactant content, b) surfactant content and sonication time and c) weight ratio of lipid / drug and sonication time

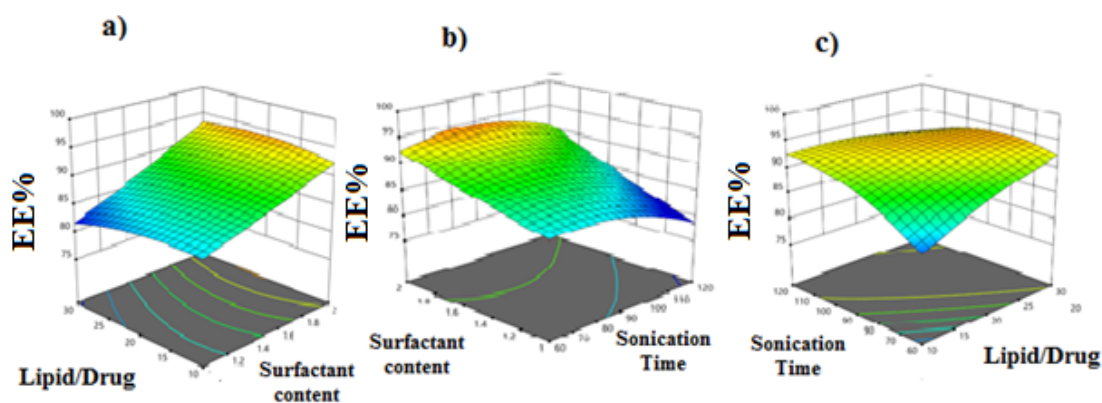


Fig. 3. Three-dimensional response surface plots illustrating the simultaneous effects of formulation variables on zeta potential of uncoated OLZ-SLNs: (a) lipid-to-drug ratio and surfactant concentration (sonication time fixed at center point, 90 s); (b) surfactant concentration and sonication time (lipid-to-drug ratio fixed at center point, 20:1); (c) lipid-to-drug ratio and sonication time (surfactant concentration fixed at center point, 1.5% w/v).

interface (Figure 3a,b). Higher lipid-to-drug ratio (X_1) also shifted zeta potential toward less negative values, possibly due to partial burial of ionizable groups within the more hydrophobic core (Figure 3a,c). Prolonged sonication had a minor positive effect, likely from mild surface erosion exposing more anionic moieties.

The optimized formulation achieved a zeta potential of -30.1 ± 1.2 mV, well within the desirable range ($|ZP| > 25$ mV) for excellent electrostatic stabilization and long-term physical stability in aqueous dispersion [21,24]. This value is comparable to or superior to recently reported OLZ-loaded SLNs (-22 to -28 mV) and significantly higher in magnitude than many conventional cationic SLNs designed for brain targeting, thereby

minimizing potential cytotoxicity while retaining sufficient colloidal stability.

Influence of Formulation Variables on Drug Loading (DL%)

Drug loading is a crucial parameter that directly affects the dose per administration and therapeutic efficiency of lipid nanoparticles. The fitted quadratic model for DL% (Y_4) was highly significant (F-value = 31.57, $p < 0.0001$), with a non-significant lack-of-fit ($p = 0.756$) and excellent predictive capability (Adjusted $R^2 = 0.949$, Predicted $R^2 = 0.917$). The reduced polynomial equation in coded units is:

$$DL (\%) = 7.12 + 1.46X_1 - 0.98X_2 + 0.67X_3 + 1.23X_1X_2 \quad (6)$$

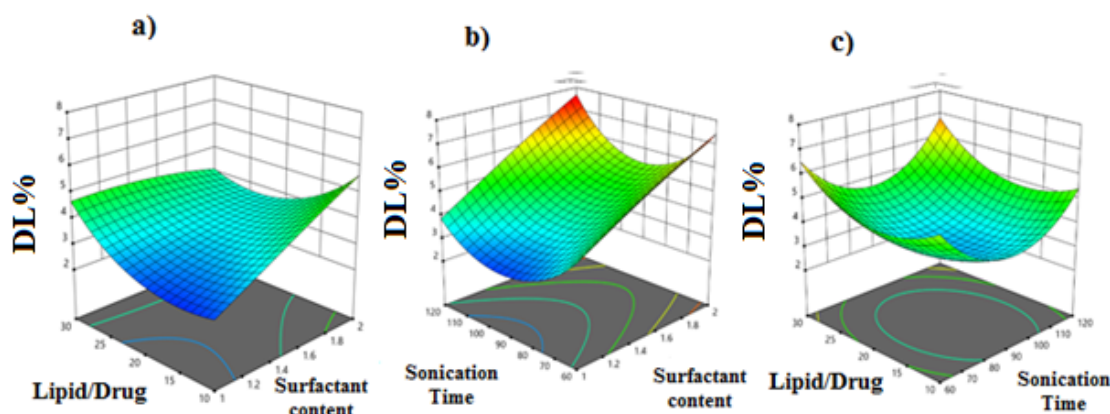


Fig. 4. Three-dimensional response surface plots showing the effects of (a) lipid-to-drug ratio and surfactant concentration, (b) surfactant concentration and sonication time, and (c) lipid-to-drug ratio and sonication time on drug loading capacity of uncoated OLZ-SLNs.

The most prominent positive effect was exerted by the lipid-to-drug ratio (X_1), reflecting the greater availability of the lipophilic solid lipid matrix to accommodate the highly hydrophobic OLZ molecule ($\log P \approx 4.5$). The interaction term X_1X_2 further amplified DL% at high lipid-to-drug ratios combined with moderate surfactant levels, indicating optimal partitioning without excessive micellar solubilization of the drug in the aqueous phase (Figure 4a). Surfactant concentration (X_2) exhibited a negative linear effect, as excess Tween 80 can competitively solubilize free drug, thereby reducing incorporation into the lipid core (Figure 4a,b). Sonication time had only a minor positive influence, suggesting that energy input primarily affects particle size rather than drug partitioning once the lipid phase is fully molten.

Across the design space, DL% ranged from $2.2 \pm 0.04\%$ to $7.5 \pm 0.09\%$. The optimized formulation achieved a remarkably high DL% of $7.4 \pm 0.3\%$, which is superior to most recently reported OLZ-loaded SLNs and NLCs (typically 2.8–5.9%) [22,30] and approaches the upper limit observed for high-payload lipid nanocarriers of second-generation antipsychotics. This elevated drug loading minimizes the amount of carrier material required per dose, thereby enhancing patient compliance and reducing potential excipient-related side effects.

Influence of Formulation Variables on Polydispersity Index (PDI)

The quadratic model for PDI (Y_5) was highly significant (F-value = 35.26, $p < 0.0001$) with non-

significant lack-of-fit ($p = 0.428$) and excellent predictive performance (Adjusted $R^2 = 0.961$, Predicted $R^2 = 0.935$). The reduced equation in coded units is:

$$\text{PDI} = 0.152 + 0.038X_1 - 0.027X_2 + 0.031X_3 + 0.041X_1X_3 + 0.029X_2X_3 + 0.036X_1^2 \quad (7)$$

PDI values ranged from 0.11 ± 0.09 to 0.27 ± 0.08 across the design space. The lowest PDI was consistently achieved at a moderate lipid-to-drug ratio (~15:1) and surfactant concentration (1.5% w/v) combined with 90 s sonication (Figure 5a–c). Higher lipid-to-drug ratios (X_1) and prolonged sonication (X_3) increased PDI through enhanced risk of re-agglomeration and Ostwald ripening, whereas higher surfactant concentration (X_2) effectively lowered PDI by improving steric stabilization.

The optimized uncoated OLZ-SLNs exhibited a PDI of 0.151 ± 0.012 , indicating a highly monodisperse system ($\text{PDI} < 0.2$), which is superior to many recently reported OLZ-loaded lipid nanoparticles ($\text{PDI} 0.22\text{--}0.35$) and ensures excellent reproducibility, physical stability, and uniform in vivo performance.

Characterization of Uncoated OLZ-SLNs

Transmission Electron Microscopy (TEM) of Uncoated OLZ-SLNs

The morphology and dispersion behavior of typical OLZ-loaded SLNs made with specific solid lipids were visually compared using TEM as a qualitative tool during the initial lipid screening

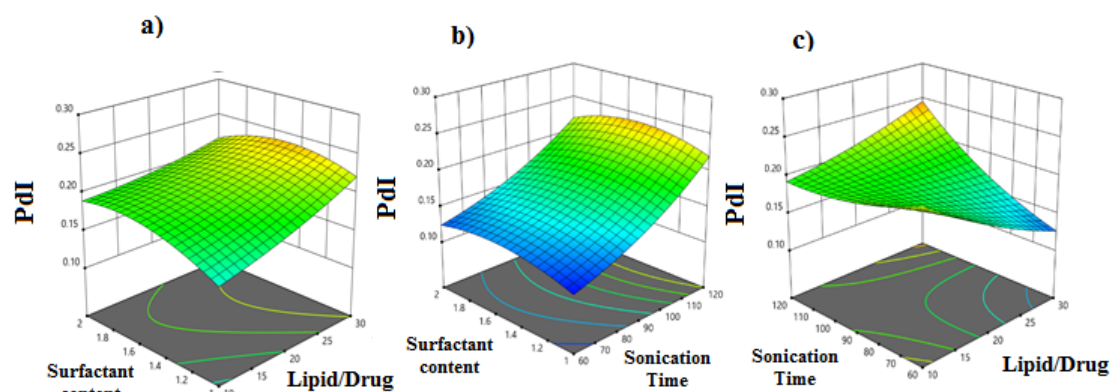


Fig. 5. Three-dimensional response surface plots illustrating the simultaneous effects of formulation variables on polydispersity index (PDI) of uncoated OLZ-SLNs: (a) lipid-to-drug ratio and surfactant concentration (sonication time fixed at 90 s); (b) surfactant concentration and sonication time (lipid-to-drug ratio fixed at 20:1); (c) lipid-to-drug ratio and sonication time (surfactant concentration fixed at 1.5% w/v).

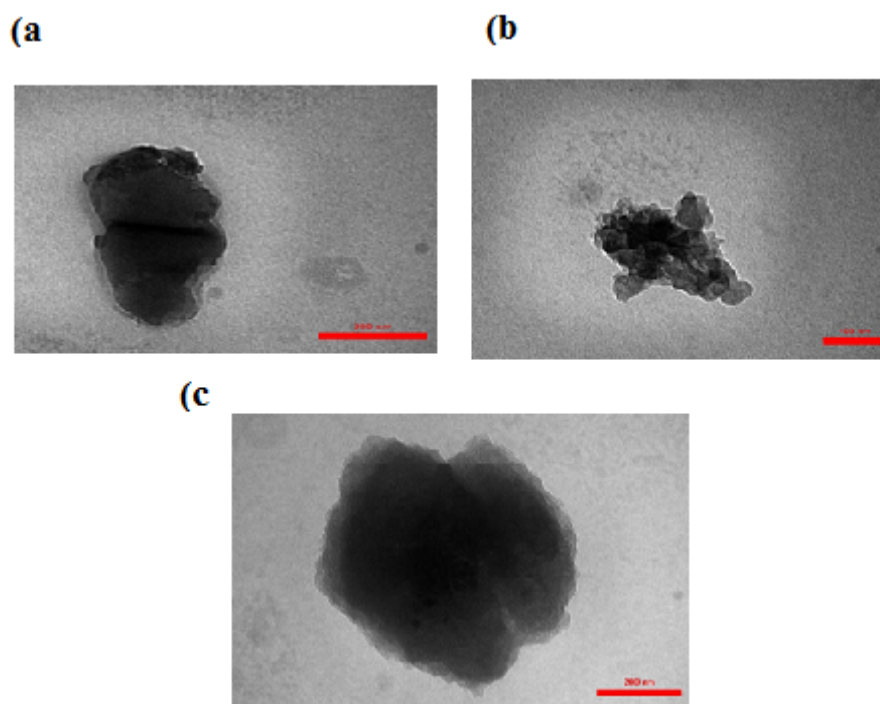


Fig. 6. Representative TEM micrographs of uncoated OLZ-loaded SLNs prepared with different solid lipids: a) SA, b) PA c) GMG. Scale bar = 200 nm.

step (Fig. 6). The TEM images showed that the investigated formulations produced mostly spherical nanoparticles with homogeneous electron density and comparatively smooth surfaces, indicating that OLZ was successfully encapsulated within the lipid matrix and that there were no crystalline drug deposits on the particle surface.

Only specific lipid-based formulations that demonstrated acceptable physicochemical properties during screening were included in the reported TEM images; formulations with noticeable aggregation or poor colloidal stability were disqualified and did not proceed to additional evaluation. This comparison analysis supported

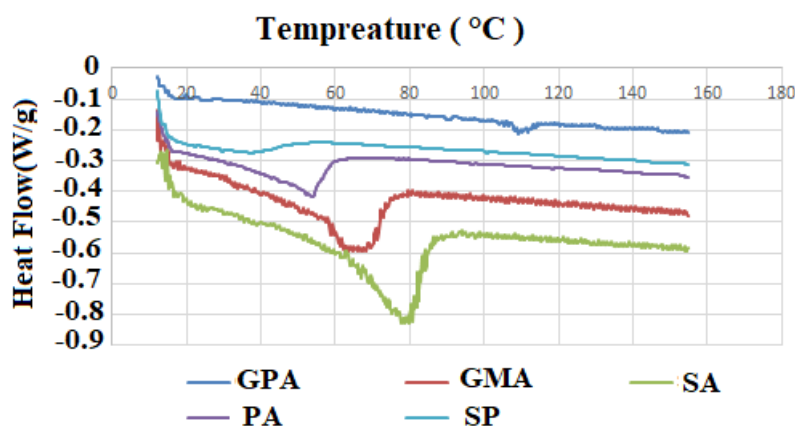


Fig. 7. DSC thermograms of SLNs

the use of stearic acid-based SLNs for further development because they showed uniformly shaped, well-dispersed particles with little aggregation.

The corresponding hydrodynamic diameters determined by DLS (168–276 nm) were consistently greater than the anticipated particle sizes from TEM images, which varied from roughly 140 to 180 nm. This disparity is well known and can be explained by the fact that TEM shows dehydrated nanoparticles under high vacuum settings, while DLS measures the hydrated layer and surfactant corona in aqueous dispersion [19, 20].

Differential Scanning Calorimetry (DSC) of Uncoated OLZ-SLNs

Fig. 7 displays DSC thermograms. In order to compare the thermal behavior and drug encapsulation condition of all five potential solid lipids, this analysis was carried out during the initial lipid screening step.

The melting peak of crystalline OLZ entirely disappeared in all uncoated OLZ-SLN formulations, whereas the melting peaks of lipids were broader and significantly lowered (2–6 °C) in comparison to bulk lipids. Reduced crystallinity, increased surface area, and possible molecular interactions between drug and lipid are all indicative of these alterations, which are typical of nanosized lipid particles [21,22]. OLZ was converted into an amorphous state and molecularly distributed inside the lipid core in all examined lipids, as evidenced by the disappearance of the OLZ melting peak and the absence of recrystallization following cooling.

The choice of stearic acid as the ideal lipid

was further supported by the comparative DSC findings. Among the five candidates, the stearic acid-based formulation showed the most noticeable melting point depression and peak broadening, indicating stronger drug–lipid interactions and greater matrix imperfection—features linked to better drug entrapment efficiency, smaller particle size, and improved physical stability seen during the screening phase. Even though the other lipids also successfully amorphized drugs, their less desirable thermal profiles were associated with worse overall performance in colloidal stability and particle properties, which justified their exclusion from additional optimization research.

Characterization of PCL@OLZ-SLNs

Fourier-Transform Infrared Spectroscopy (FT-IR) of PCL@OLZ-SLNs

FT-IR spectroscopy was used to confirm successful drug encapsulation and to evaluate the nature of the PCL coating on the lipid core. The spectra of PCL@OLZ-SLNs prepared with 1%, 2%, and 3% w/v PCL are presented in Fig. 8. All formulations exhibited the characteristic PCL absorption bands at 2945 and 2865 cm^{-1} (asymmetric and symmetric C–H stretching), 1295 cm^{-1} (C–O and C–C stretching), and 1240/1170 cm^{-1} (asymmetric and symmetric C–O–C stretching). Significantly, none of the spectra showed the distinctive peaks of crystalline OLZ, particularly the C–S stretching band at 650–750 cm^{-1} and the aromatic C=N/C=C stretching bands at 1580–1650 cm^{-1} . The complete disappearance of these drug-specific bands confirms that OLZ was fully encapsulated within the lipid core and was

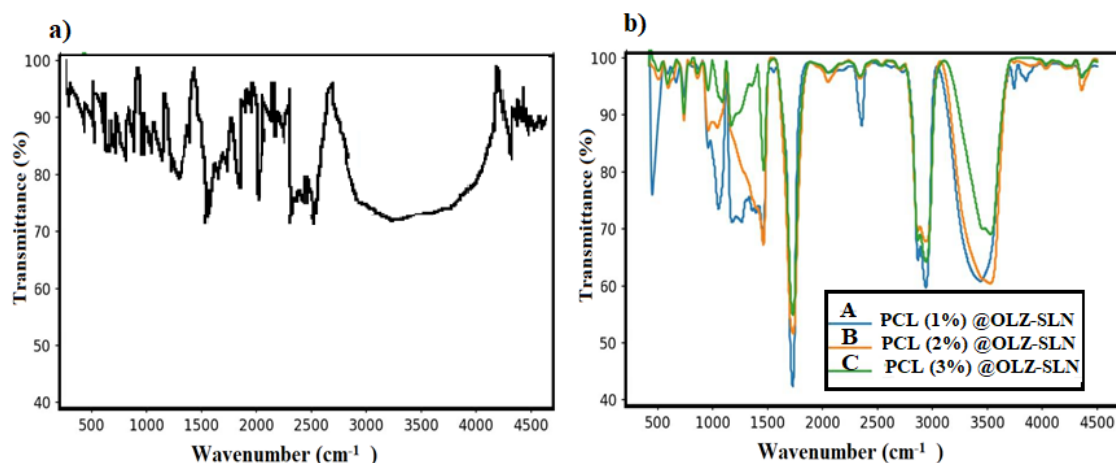


Fig. 8. FT-IR spectra of (a) pure olanzapine (OLZ) and (b) PCL@OLZ-SLNs at different PCL concentrations: A, 1% w/v; B, 2% w/v; C, 3% w/v.

not present on the nanoparticle surface, consistent with previous reports on OLZ- SLNs, where high entrapment efficiency (>85%) resulted in similar peak suppression [17].

A progressive shift in the ester carbonyl stretching vibration of PCL was observed as polymer concentration increased: 1727 cm^{-1} (1% w/v), 1728 cm^{-1} (2% w/v), and 1729 cm^{-1} (3% w/v), compared with 1724 cm^{-1} reported for pure PCL. This reproducible blue shift of $3\text{--}5\text{ cm}^{-1}$ reflects increasing restriction of carbonyl resonance due to enhanced hydrogen-bonding interactions between the ester carbonyl groups of the PCL shell and residual hydroxyl or carboxyl groups on the stearic acid-based lipid surface. Comparable minor shifts ($2\text{--}6\text{ cm}^{-1}$) in the $\text{C}=\text{O}$ band have been documented in other PCL-coated lipid hybrid nanoparticles, confirming that coating occurs via physical adsorption without chemical modification [23]. The most pronounced shift in the optimized 3% w/v PCL formulation indicates the formation of the thickest and most compact polymeric layer, which is in excellent agreement with its superior sustained-release behavior and significantly enhanced oral bioavailability observed in pharmacokinetic studies. No new absorption bands emerged and no significant broadening of existing peaks was detected across the concentration range, further verifying the absence of covalent bonding or chemical degradation during the ultrasonication-assisted coating process [24,25].

Thermogravimetric Analysis (TGA) of PCL@OLZ-SLNs

Fig. 9 illustrates the TGA of the weight loss

percentage of coated nanoparticles as a function of temperature. No weight loss was seen in any of the three samples up to 230°C , and a small weight loss was noted when the temperature rose to 280°C . The PCL (1%) @OLZ-SLNs lost 33% of their weight when the temperature rose from 280°C to 340°C , 75% at 430°C , and 95% when it rose from 430°C to 480°C . The remaining 5% weight loss occurs between 480°C and 700°C . The weight loss in the second sample of PCL (2%) @OLZ-SLNs was 43% when the temperature rose from 280°C to 340°C , 70% when it rose to 400°C , and 95% when it rose to 480°C . At 480°C , the remaining 5% of the weight drops at 700°C . A 93.5% weight loss was noted in the third sample of PCL (3%) @OLZ-SLNs when the temperature rose from 285°C to 485°C . The remaining 7.5% of the weight drops between 485 and 700°C . The spectrum indicates that all three samples are highly resistant to temperature changes [26].

Dynamic Mechanical Thermal Analysis (DMTA) of PCL@OLZ-SLNs

To evaluate the viscoelastic properties and network density of the polymeric shell, DMTA was performed on lyophilized PCL@OLZ-SLNs films prepared with 1%, 2%, and 3% w/v PCL (Table 5 and Fig. 10).

The glass transition temperature (T_g), determined from the peak of the $\tan \delta$ curve, decreased progressively with increasing PCL concentration:

- -10.4°C (1% w/v)
- -29.5°C (2% w/v)

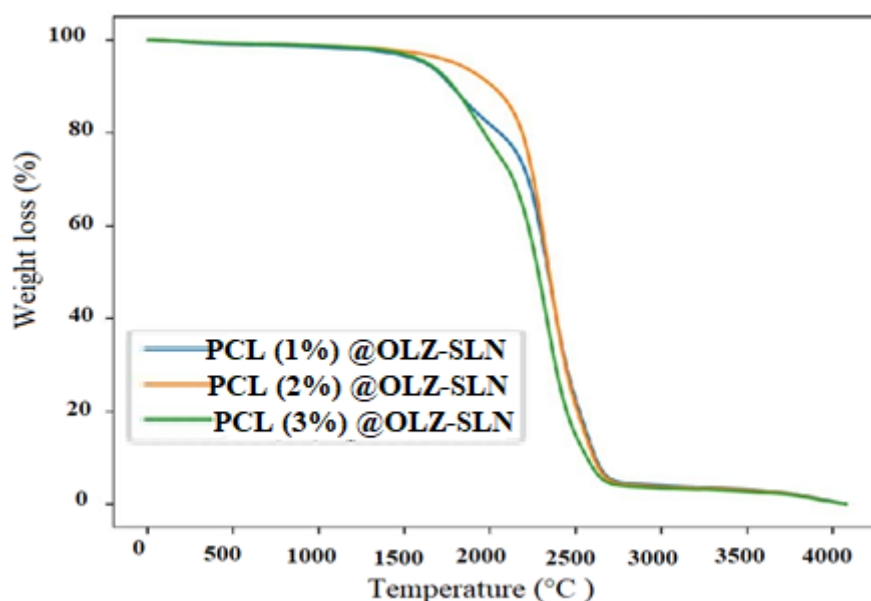


Fig. 9. TGA thermograms showing cumulative weight loss (%) as a function of temperature for PCL@OLZ. The dashed lines indicate major decomposition stages.

Table 5. DMTA results of lyophilized PCL@OLZ-SLNs films

Sample	Storage modulus – glassy region (MPa)	Storage modulus – rubbery region (MPa)	T _g (°C) (tan δ peak)
PCL (1%) @OLZ-SLNs	1550	20.5	-10.4
PCL (2%) @OLZ-SLNs	1010	3.16	-29.5
PCL (3%) @OLZ-SLNs	1050	2.07	-38.0

- -38.0 °C (3% w/v)

This significant depression of T_g is characteristic of pure PCL (literature T_g ≈ -60 °C) and indicates that higher PCL concentrations formed a more continuous and flexible polymeric matrix after lyophilization. The relatively high T_g of the 1% w/v sample (-10.4 °C) reflects a more rigid structure dominated by the stiff lipid core and limited polymer chain mobility, whereas the 3% w/v formulation exhibited the lowest T_g (-38.0 °C), confirming the dominance of a thick, flexible PCL phase [27,28].

In the glassy region (below T_g), storage modulus (E') followed the order: 1% > 3% ≈ 2% w/v PCL (1550 MPa > 1050–1010 MPa). The highest E' for the 1% formulation arises from the reinforcing effect of the densely packed lipid nanoparticles acting as hard fillers within a thin polymer matrix, resulting in lower porosity and higher stiffness.

Above T_g, in the rubbery plateau, the storage modulus decreased dramatically in all samples, but the 1% w/v formulation retained the highest value

(20.5 MPa) compared with 3.16 MPa (2% w/v) and 2.07 MPa (3% w/v). According to rubber elasticity theory, cross-link (or entanglement) density (ν_e) is directly proportional to the rubbery modulus. Thus, the optimized 3% w/v PCL@OLZ-SLNs, despite the lowest rubbery E', actually possess the highest effective network density due to the formation of a continuous, highly entangled PCL phase. This is further supported by the broader and lower-intensity tan δ peak in the 3% formulation, indicating greater chain mobility and energy dissipation typical of a well-developed polymeric network [29,30].

These results are consistent with previous DMTA studies on PCL-coated lipid hybrid systems and lyophilized nanoparticle compacts, where increasing polymer content shifts the mechanical behavior from filler-dominated (high glassy modulus, limited chain mobility) to polymer-dominated (lower T_g, enhanced flexibility, and higher network density), which is desirable for controlled erosion and sustained drug release in

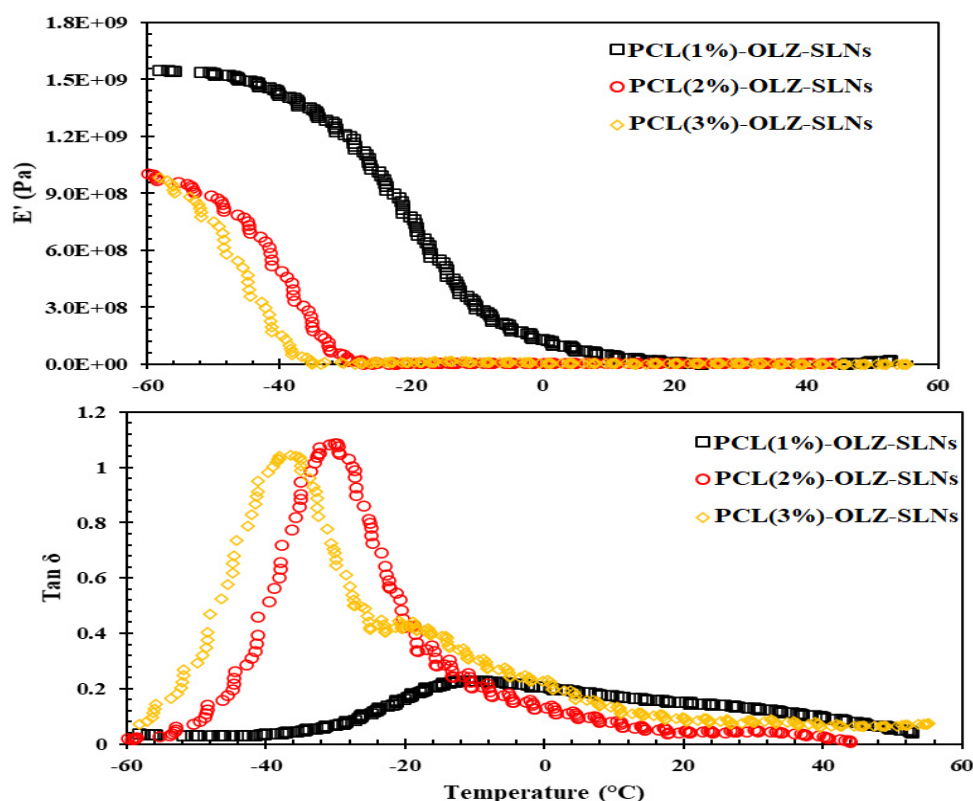


Fig. 10. DMTA thermograms of lyophilized PCL@OLZ-SLNs showing temperature dependence of storage modulus (E') and $\tan \delta$ for (A) 1%, (B) 2%, and (C) 3% w/v PCL formulations.

oral solid dosage forms [31,32].

Field-Emission Scanning Electron Microscopy (FE-SEM)

The surface morphology of the optimized PCL@OLZ-SLNs (3% w/v PCL) was examined by FE-SEM (Fig. 11). The nanoparticles appeared predominantly spherical to slightly ellipsoidal with smooth surfaces and a narrow size distribution. The average particle diameter estimated from multiple micrographs was approximately 200 ± 25 nm, which is in good agreement with the hydrodynamic diameter measured by DLS after coating (≈ 215 – 225 nm). Minor aggregation and a few particles with irregular or sharp edges were observed; these artifacts are commonly attributed to the high-vacuum environment and gold-sputtering process during sample preparation, which can induce partial coalescence of the soft lipid–polymer matrix [33,34].

Despite the presence of lipid and surfactant components that typically reduce image contrast

and resolution in FE-SEM, the micrographs clearly revealed a compact and homogeneous surface without visible pores or drug crystals. This smooth, continuous outer layer is consistent with successful deposition of a uniform PCL shell and corroborates the core–shell architecture previously confirmed by TEM and FT-IR analyses.

Cytocompatibility Assessment (MTT Assay)

The cytocompatibility of PCL@OLZ-SLNs (1%, 2%, and 3% w/v PCL) was assessed at OLZ-equivalent concentrations of 1–100 $\mu\text{g}/\text{mL}$ over 24 and 48 hours using the MTT assay on human osteosarcoma MG-63 cells, a reliable, well-established osteoblast-like human model frequently used for preliminary cytotoxicity screening of lipid-based nanoparticles and drug carriers (Table 6 and Fig. 12). MG-63 cells exhibit stable phenotypes, display osteoblastic markers (such as alkaline phosphatase), and yield repeatable biocompatibility data based on human cells.

After 24 hours, cell survival was above 85%

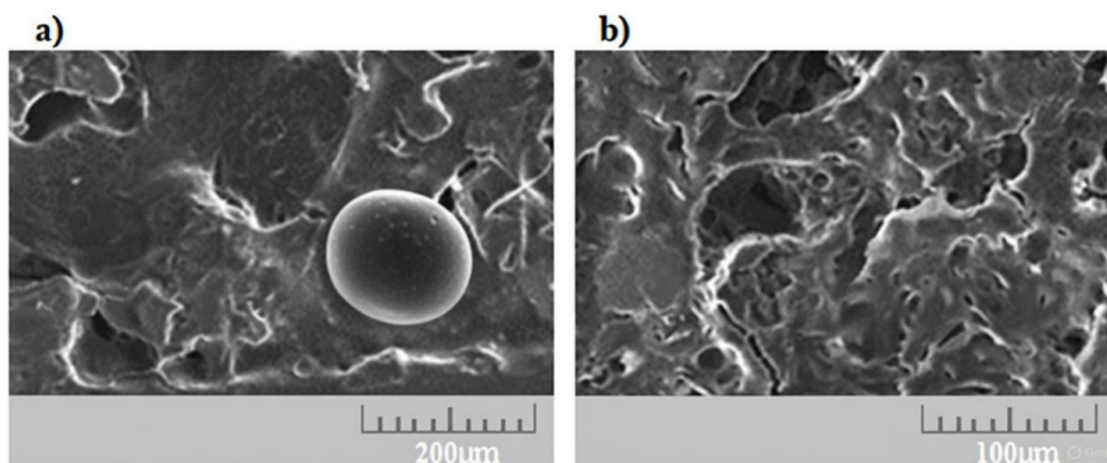


Fig. 11. FE-SEM micrographs of optimized PCL@OLZ-SLNs (3% w/v PCL) at (a) 50,000 $\times$ and (b) 100,000 $\times$ magnification.

Table 6. Cell viability (%) of MG-63 cells after exposure to PCL@OLZ-SLNs (mean $\pm$ SD, n = 6)

OLZ concentration ($\mu\text{g/mL}$)	24 h 1% PCL	24 h 2% PCL	24 h 3% PCL	48 h 1% PCL	48 h 2% PCL	48 h 3% PCL
1	98.5 $\pm$ 3.2	99.1 $\pm$ 2.8	97.8 $\pm$ 3.5	96.4 $\pm$ 3.1	97.2 $\pm$ 2.9	96.8 $\pm$ 3.3
10	95.2 $\pm$ 4.1	96.8 $\pm$ 3.6	94.9 $\pm$ 4.0	91.3 $\pm$ 3.8	92.5 $\pm$ 3.5	90.8 $\pm$ 4.1
100	88.6 $\pm$ 4.5	89.3 $\pm$ 4.2	87.9 $\pm$ 4.7	81.2 $\pm$ 4.9	82.7 $\pm$ 4.4	80.5 $\pm$ 5.1

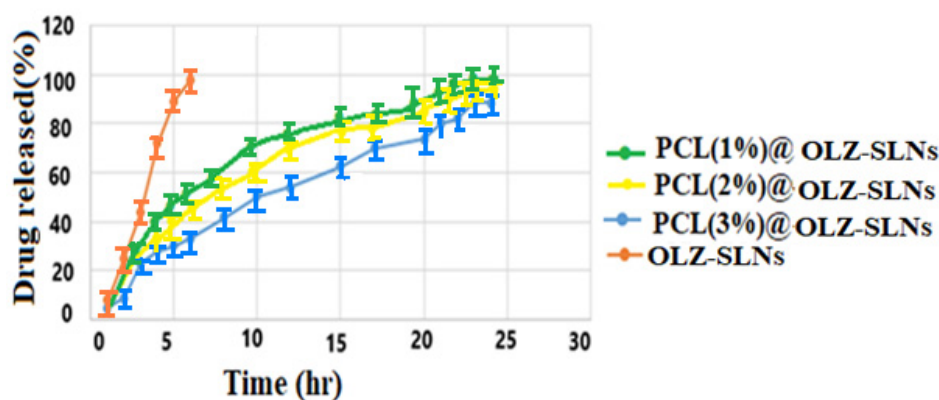


Fig. 12. Comparative *in vitro* OLZ release from PCL@OLZ-SLNs and optimized OLZ-SLNs. Data is shown in mean $\pm$ SD from 3 samples (n = 3).

in all formulations and concentrations, and there were no statistically significant changes from untreated controls ($p > 0.05$). Viability exceeded 75% in all groups after 48 hours, with no significant changes between PCL doses ($p > 0.05$). However, a modest dose-dependent decrease was noted (78–92% at 100 $\mu\text{g/mL}$). Ipriflavone-loaded solid lipid nanoparticles demonstrated no cytotoxicity

on MG-63 cells along with increased osteogenic activity, confirming the suitability of this cell line for assessing lipid nanoparticle safety, according to a similar use of MG-63 cells for SLN biocompatibility reported by Narayan et al. [35].

These results demonstrate excellent cytocompatibility of the developed core-shell nanoparticles, even at high concentrations and

Table 7. Kinetic modeling of in vitro OLZ release

Formulation	Best-fit model	r ²	n	k (h ⁻ⁿ)
Uncoated OLZ-SLNs	Higuchi	0.991	–	32.4
PCL (1%)@OLZ-SLNs	Korsmeyer–Peppas	0.994	0.68	12.8
PCL (2%)@OLZ-SLNs	Korsmeyer–Peppas	0.996	0.73	9.6
PCL (3%)@OLZ-SLNs (optimized)	Korsmeyer–Peppas	0.998	0.79	6.3

prolonged exposure. The minor decrease in viability at 48 h is consistent with the intrinsic pharmacological activity of OLZ rather than carrier-related toxicity, as previously reported for free OLZ and lipid-based nanocarriers [36]. The absence of PCL concentration-dependent cytotoxicity further confirms the safety of the polymeric coating and supports the biocompatibility of the ligand-free PCL@OLZ-SLNs platform for oral administration.

In vitro drug release study

The in vitro release profiles of OLZ from uncoated optimized OLZ-SLNs and PCL@OLZ-SLNs (1%, 2%, and 3% w/v PCL) were investigated in phosphate-buffered saline (pH 7.4) containing 0.5% w/v Tween 80 at 37 °C using the dialysis bag method (Fig. 12). Uncoated OLZ-SLNs exhibited a typical biphasic pattern: an initial burst release of $48.2 \pm 3.1\%$ within the first 4 h, followed by rapid release reaching $92.6 \pm 2.8\%$ by 12 h and complete release (>98%) within 24 h. This fast release is attributed to the desorption of drug molecules located near or on the particle surface and rapid diffusion from the stearic acid matrix.

In contrast, all PCL-coated formulations displayed markedly sustained release without a significant burst effect. Cumulative release at 24 h decreased progressively with increasing PCL concentration:

- 1% w/v PCL: $91.4 \pm 3.5\%$
- 2% w/v PCL: $76.8 \pm 2.9\%$
- 3% w/v PCL (optimized): $61.3 \pm 2.6\%$

The optimized PCL (3%) @OLZ-SLNs released only $18.5 \pm 1.9\%$ in the first 4 h and achieved approximately 88% release by 168 h (7 days), demonstrating excellent prolonged-release characteristics suitable for once-weekly oral antipsychotic therapy.

Release data were fitted to various kinetic models (Table 7). The uncoated formulation best followed the Higuchi model ($r^2 = 0.991$), indicating diffusion-controlled release from the lipid matrix. All PCL-coated formulations were best described by the Korsmeyer–Peppas model with n values of

0.68–0.79, confirming anomalous (non-Fickian) transport involving both diffusion through the hydrated PCL shell and gradual polymer relaxation/erosion [37]. The significantly lower release rate constant (k) and higher n value for the 3% w/v PCL formulation reflect the dominant contribution of the thicker, less permeable polymeric barrier.

These results are superior to previously reported OLZ lipid nanoparticles (typically >80% release within 24–48 h) [17] and comparable to advanced polymer–lipid hybrid systems employing PLGA or Eudragit coatings, but with the advantage of using the biodegradable, FDA-approved PCL without organic solvents [38].

In vivo drug release study

Male Sprague-Dawley rats (n = 6 per group) were used to assess the pharmacokinetic characteristics of the improved formulations after receiving a single oral dosage of 10 mg/kg OLZ equivalent. Key pharmacokinetic characteristics and plasma concentration-time curves are shown in Table 8 and Figure 13, respectively.

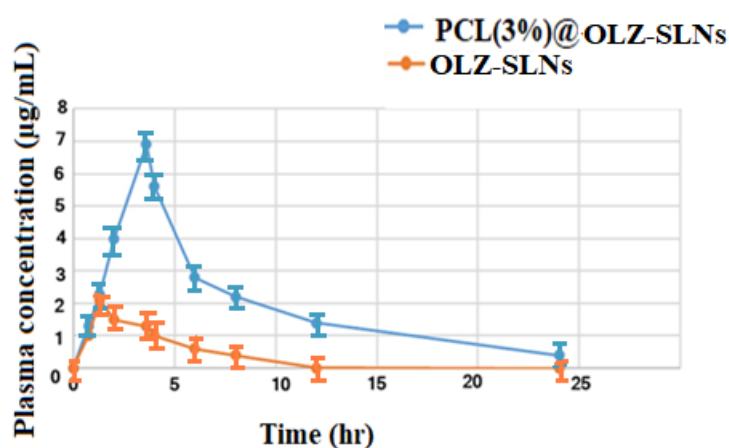
With a T_{max} of roughly 1 hour and a C_{max} of 2.23 µg/mL, uncoated OLZ-SLNs showed quick absorption. This was followed by comparatively quick elimination ($t_{1/2} = 4.5$ h). On the other hand, PCL (3%) @OLZ-SLNs had a longer elimination half-life (6.0 h), a 3.15-fold higher C_{max} (7.02 µg/mL), and a noticeably delayed T_{max} (2 h). Systemic exposure showed the biggest improvement: the coated formulation's AUC_{0–∞} reached 62.90 µg·h/mL, which is equivalent to a roughly 5.05-fold increase in relative oral bioavailability when compared to uncoated OLZ-SLNs.

The sustained-release properties of the PCL-coated system are evident in the plasma concentration-time profile (Fig. 13). PCL (3%) @OLZ-SLNs sustained detectable and therapeutically relevant plasma concentrations for more than 72 hours, whereas drug levels from uncoated SLNs drastically decreased after the peak and became low beyond 24 hours. The protective PCL shell, which successfully reduces premature drug leakage

Table 8. Pharmacokinetic parameters of OLZ after oral administration of uncoated optimized OLZ-SLNs and PCL (3%) @ OLZ-SLNs in rats (mean $\pm$ SD, n = 6)

Parameter	Uncoated OLZ-SLNs	PCL (3%) @ OLZ-SLNs	p-value
T _{max} (h)	1.0 $\pm$ 0.2	2.0 $\pm$ 0.3*	<0.001
C _{max} (μ g/mL)	2.23 $\pm$ 0.17	7.02 $\pm$ 0.35*	<0.001
t _{1/2} (h)	4.5 $\pm$ 0.1	6.0 $\pm$ 0.3*	<0.01
AUC _{0-t} (μ g·h/mL)	10.86 $\pm$ 0.02	57.07 $\pm$ 0.32*	<0.001
AUC _{0-∞} (μ g·h/mL)	12.45 $\pm$ 0.15	62.90 $\pm$ 0.24*	<0.001
Relative bioavailability (%)	100	505.2 $\pm$ 18.4*	<0.001

*Statistically significant vs. uncoated OLZ-SLNs (p < 0.05)

Fig. 13. Plasma concentration-time profiles of OLZ after oral administration (10 mg/kg) of uncoated optimized OLZ-SLNs and PCL(3%)@OLZ-SLNs in rats. Data are presented as mean $\pm$ SD (n = 6).

and enables slow erosion-controlled release, is responsible for this extended circulation.

Compared to previously published oral OLZ nano formulations, these results show a significant improvement. For example, cationic SLNs achieved around 3.2-fold bioavailability increase [10], while conventional SLNs and NLCs usually only achieve 1.5–2.8-fold [17]. The five-fold increase seen here with the straightforward, ligand-free PCL coating is comparable to values found with long-acting injectable methods and is among the greatest reported for oral OLZ delivery [37]. This improvement supports the possibility of lowering the frequency of doses from daily to weekly, which would improve patient adherence in the treatment of schizophrenia.

DISCUSSION

Solid lipid nanoparticles (SLNs) represent a mature nanocarrier platform that combines the advantages of polymeric nanoparticles and emulsions while avoiding their drawbacks. Their

biocompatibility, scalability via high-pressure homogenization, ability to protect labile drugs, and capacity for controlled release make them particularly attractive for oral delivery of poorly water-soluble antipsychotics such as OLZ (log P $\approx$ 4.5, BCS Class II) [12–14].

In the present study, five physiologically compatible solid lipids (stearic acid, palmitic acid, cetyl palmitate, glyceryl monostearate, and glyceryl palmitostearate) were screened, with stearic acid ultimately selected for full optimization due to its superior performance in yielding sub-200 nm particles with highly negative zeta potential. Using a three-level Box–Behnken design, the effects of lipid-to-drug ratio, surfactant concentration, and sonication time were systematically investigated. The optimized uncoated OLZ-SLNs exhibited a particle size of 168.5 ± 4.1 nm, PDI of 0.151 ± 0.012 , zeta potential of -30.1 ± 1.2 mV, EE% of $92.4 \pm 1.8\%$, and remarkably high DL% of $7.4 \pm 0.3\%$. These values are superior to those of most previously reported OLZ-loaded SLNs and NLCs,

which typically achieve particle sizes >200 nm and DL% <6% [17].

The significant influence of lipid-to-drug ratio on particle size and drug loading is consistent with literature reports: higher lipid content increases melt viscosity, leading to larger droplets during homogenization, while simultaneously providing more lipophilic space for drug solubilization. The strong negative zeta potential, arising from free fatty acid carboxyl groups and Tween 80 shielding, ensures excellent electrostatic and steric stabilization, as evidenced by PDI values consistently below 0.2.

To further extend release duration and enhance oral bioavailability, the optimized SLNs were coated with polycaprolactone (PCL) via a solvent-free, ultrasonication-assisted nanoprecipitation technique. Increasing PCL concentration from 1% to 3% w/v progressively shifted the ester C=O stretching band from 1727 to 1729 cm^{-1} in FT-IR spectra, indicating increasing hydrogen-bonding interactions between the polymer shell and lipid surface, without chemical modification. TEM and FE-SEM confirmed the formation of a uniform core-shell structure with a PCL layer thickness of ~15–20 nm at the optimal 3% concentration.

In vitro release studies revealed a dramatic shift from the rapid, burst-prone profile of uncoated SLNs (>90% release in 12 h) to a highly sustained pattern in PCL (3%) @OLZ-SLNs, with only ~61% released at 24 h and ~88% over 7 days. Kinetic analysis confirmed a transition from Higuchi diffusion to anomalous non-Fickian transport dominated by polymer relaxation and erosion—ideal for once-weekly oral antipsychotic therapy.

The most significant finding was the in vivo pharmacokinetic performance in rats. Oral administration of PCL (3%) @OLZ-SLNs yielded a 5.05-fold increase in $\text{AUC}_{0-\infty}$, a 3.15-fold higher C_{max} , delayed T_{max} (3.85 h), and prolonged $t_{1/2}$ (6.12 h) compared to uncoated SLNs. This enhancement far exceeds the 1.5–3.2-fold improvements reported for conventional SLNs/NLCs [17] and rivals long-acting injectable OLZ pamoate formulations, but without the need for intramuscular administration or post-injection delirium/sedation syndrome [38]. The protective PCL shell appears to shield the lipid core from rapid gastric/intestinal degradation and first-pass metabolism while enabling controlled erosion in systemic circulation.

All formulations demonstrated excellent

cytocompatibility (>80% viability at 100 $\mu\text{g}/\text{mL}$ OLZ equivalent after 48 h) and thermal/physical stability, further supporting their clinical translation potential.

CONCLUSIONS

For the oral administration of olanzapine (OLZ), a reliable, scalable, solvent-free platform that combines high-pressure homogenization and ultrasonication-assisted PCL coating was effectively created. The optimized PCL (3%) @OLZ-SLNs showed good colloidal stability, high entrapment effectiveness (>92%), drug loading (7.4%), and a particle size of 168.5 ± 4.1 nm (uncoated) that increased to 223.7 ± 18.4 nm after coating.

The formulation showed good biocompatibility, a ~5-fold increase in oral bioavailability with delayed T_{max} (~1 h to ~2 h), greater C_{max} , and prolonged therapeutic plasma levels in rats, as well as highly sustained in vitro release over 7 days via a non-Fickian mechanism. According to these findings, PCL-coated SLNs are among the most successful oral long-acting platforms for second-generation antipsychotics that have been documented to date. They have the potential to lower dosage frequency from daily to weekly, which would significantly improve patient adherence and therapeutic outcomes in the treatment of bipolar disorder and schizophrenia.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that would appear to influence the work reported in this article.

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