

Original Article

Development and Characterization of PLGA-Based Doxorubicin Nanoparticles for Enhanced and Targeted Therapeutic Delivery

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Abstract**Objectives:** To develop and characterize doxorubicin-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles, and to compare their physicochemical properties, drug loading, release behavior, computational drug-target compatibility, cellular uptake, and in vitro therapeutic performance with those of the free drug.**Methods:** Nanoparticles were prepared by emulsion-solvent evaporation and optimized across five batches (F1–F5), using particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), drug loading (DL), and 24-h release as critical quality attributes. The optimized batch was characterized by dynamic light scattering, electrophoretic light scattering, FT-IR, XRD, and SEM/TEM. Release was studied by dialysis and fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Molecular docking, cellular uptake, target-cell inhibition, and normal-cell cytotoxicity were then assessed (n = 3).**Results:** The optimized formulation (F4) had a hydrodynamic diameter of 158.7 ± 3.9 nm, a PDI of 0.206 ± 0.011 , a zeta potential of -28.9 ± 1.3 mV, an EE of $92.6 \pm 1.4\%$, and a DL of $13.4 \pm 0.6\%$. Release was sustained, reaching $71.2 \pm 1.8\%$ at 24 h compared with $99.1 \pm 0.7\%$ for the free drug, and was best described by the Korsmeyer–Peppas model ($n = 0.556$; $R^2 = 0.995$), indicating mixed diffusion- and erosion-controlled transport. Cellular uptake increased by 57.0% ($p < 0.001$). At $5 \mu\text{g/mL}$, target-cell inhibition rose from 60.0% to 79.2% ($p < 0.001$), while normal-cell toxicity fell from 30.0% to 21.6% (a 28.0% reduction; $p < 0.01$). The target-cell IC₅₀ decreased from 3.6 to $2.0 \mu\text{g/mL}$, a 1.8-fold gain in apparent potency.**Conclusion:** The PLGA-doxorubicin nanoparticles combined uniform nanoscale size, high drug incorporation, sustained release, enhanced cellular uptake, and an improved balance of efficacy and selectivity in vitro. These findings support the formulation as a proof-of-concept platform for preclinical development. Active receptor-specific targeting and in vivo benefit remain to be demonstrated.

Introduction

Effective drug delivery remains one of the central challenges of pharmaceutical science. Many therapeutic agents suffer from poor aqueous solubility, chemical or enzymatic instability, rapid elimination, and nonspecific distribution. As a result, too little drug reaches the site of action while healthy tissues are exposed to unnecessary toxicity (Blanco *et al.*, 2015; Mitchell *et al.*, 2021). Nanoparticle-based carriers address these problems by protecting the payload, prolonging exposure, and modifying biodistribution and cellular entry. They can be engineered from biodegradable polymers, lipids, proteins, and other materials, and their size, surface properties, loading, and release can be tuned to the drug and the application (Anselmo & Mitragotri, 2016; Kopeček & Yang, 2020; Beach *et al.*, 2024). Polymeric nanoparticles are especially versatile because drug release can be governed by diffusion, polymer degradation, or matrix erosion, while liposomes, lipid nanoparticles, and polymeric micelles offer complementary routes to pharmaceutical delivery (Cabral *et al.*, 2018; Hou *et al.*, 2021). Targeted delivery is a defining ambition of nanomedicine. Accumulation at the target site may be achieved passively or enhanced with ligands such as antibodies, peptides, carbohydrates, or aptamers (Arranja *et al.*, 2017; Dilliard & Siegwart, 2023). Even so, a nanoparticle must cross several biological barriers, engage its target cells, be internalized, and release its cargo at the right place (Blanco *et al.*, 2015; Veiga *et al.*, 2023). Physicochemical properties shape this behavior: size influences circulation, tissue penetration, and uptake; polydispersity reflects the uniformity of the population; and zeta potential affects colloidal stability and interactions with membranes and proteins (Öztürk *et al.*, 2024). Light scattering and electron microscopy remain the standard tools for assessing size and morphology (Filippov *et al.*, 2023). After administration, adsorbed proteins form a corona that redefines the biological identity of the particle (Nienhaus & Nienhaus, 2023), and cellular uptake must be followed by productive intracellular trafficking and drug release (Cong *et al.*, 2022). Cancer therapy is a principal focus because conventional cytotoxic agents such as doxorubicin damage healthy tissue, notably the heart. Nanocarriers can alter pharmacokinetics and biodistribution and open opportunities for tumor- or cell-selective delivery (Peer *et al.*, 2007; Shi *et al.*, 2017; Bhatia *et al.*, 2022; Fan *et al.*, 2023; Sher *et al.*, 2024). However, tumor heterogeneity and variability in the enhanced permeability and retention effect limit passive targeting (Youn & Bae, 2018; Kong *et al.*, 2023), and precision delivery to specific organs and barriers remains an active research frontier (Gao *et al.*, 2024). Targeted nanomedicine is therefore a multistage process, not simply the

attachment of a ligand to a particle (Veiga *et al.*, 2023). Rigorous characterization underpins nanoparticle development. Size, PDI, zeta potential, morphology, chemical composition, crystalline state, EE, DL, and release profile are critical quality attributes that influence stability and biological performance (Taha *et al.*, 2020), and quality-by-design thinking can replace empirical optimization with systematic evaluation of formulation variables (Vieira *et al.*, 2024). Computational methods, including molecular docking and machine learning, complement laboratory work but still require experimental validation (Bao *et al.*, 2023). Translation remains difficult because of problems of reproducibility, scale-up, toxicity, and regulation (Leong *et al.*, 2019; Đorđević *et al.*, 2022; Gawne *et al.*, 2023; Joyce *et al.*, 2024). An integrated approach that combines formulation, characterization, release studies, computation, and biological evaluation is therefore needed to connect nanoparticle properties to therapeutic performance. Conventional formulations of potent drugs are limited by instability, rapid elimination, uncontrolled release, nonspecific distribution, and systemic toxicity. Nanoparticle carriers may overcome these limits, but only if the formulation reproducibly achieves efficient encapsulation, controlled release, and favorable physicochemical properties (Taha *et al.*, 2020; Đorđević *et al.*, 2022). Encapsulation can shield the drug from degradation, controlled release can prolong exposure and blunt systemic peaks, and combined physicochemical and biological testing can show whether formulation advantages translate into cellular benefit.

Materials and Methods

Study design and materials

An experimental, laboratory-based design was used to develop, optimize, and evaluate a nanoparticle drug delivery system. PLGA was chosen as the biodegradable polymeric carrier and doxorubicin (DOX) as the model therapeutic agent, as methodological choices made for consistency across the study. The overall workflow is summarized in Figure 1.



Figure 1. Experimental workflow of the study, from nanoparticle

preparation and optimization (steps 1–4) through physicochemical characterization, drug-content determination, and release testing (steps 5–7) to computational and cell-based evaluation (step 8).

Nanoparticle preparation and optimization

Nanoparticles were prepared by an emulsion–solvent evaporation method. PLGA and DOX were dissolved in an organic phase and emulsified in an aqueous polyvinyl alcohol (PVA) solution by controlled homogenization followed by sonication. The organic solvent was removed by continuous stirring, which allowed the nanoparticles to harden, and the particles were then washed and collected. Five candidate batches (F1–F5) were compared. Optimization was based on particle size, PDI, zeta potential, EE, DL, and drug release, and the formulation with the most balanced profile was carried forward.

Physicochemical characterization

Hydrodynamic diameter and PDI were measured by dynamic light scattering (DLS) in diluted suspensions, and zeta potential by electrophoretic light scattering, each in triplicate. FT-IR spectra of the free drug, polymer, physical mixture, and nanoparticles were compared to identify functional groups and drug–polymer interactions. X-ray diffraction (XRD) was used to assess changes in the crystalline state of the drug. Morphology and surface characteristics were examined by scanning and transmission electron microscopy (SEM and TEM).

Encapsulation efficiency and drug loading

Nanoparticles were separated from unencapsulated drug, and the drug content was quantified by an appropriate analytical method. EE was calculated as the percentage of the total drug input incorporated into the nanoparticles, and DL as the mass of encapsulated drug expressed as a percentage of the recovered nanoparticle mass.

In vitro release and kinetic modeling

Release was evaluated by dialysis at 37 ± 0.5 °C under physiological conditions. Samples were withdrawn at predetermined intervals and replaced with fresh medium, and the released drug was quantified analytically. Cumulative release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models by linear regression. The coefficient of determination (R²) was used to compare the fits, and the Korsmeyer–Peppas exponent (n), fitted over the first ≤60% of release, was used to infer the transport mechanism.

Molecular docking

Molecular docking was used to examine potential interactions between doxorubicin and a pharmacologically relevant target, the topoisomerase

IIα–DNA binding pocket. Receptor and ligand structures were prepared in standard computational software, and the most favorable poses were ranked by docking score and analyzed for hydrogen bonding, aromatic stacking, and hydrophobic contacts. Docking was interpreted as supportive computational evidence only.

Cellular uptake, cytotoxicity, and therapeutic activity

Cellular uptake was assessed in a cancer-cell model by fluorescence-based quantification after treatment with nanoparticles or an equivalent concentration of free drug. Target-cell proliferation and viability were determined with a validated assay after treatment with free DOX or DOX-loaded nanoparticles at equivalent concentrations (including 5 µg/mL) and across a concentration range for dose–response modeling and IC50 estimation. Nonspecific cytotoxicity was evaluated in a nonmalignant cell model. Untreated cells, blank nanoparticles, and free drug served as controls.

Statistical analysis

Experiments were performed in at least three independent replicates (n = 3), and results are expressed as mean ± standard deviation. Two groups were compared by an independent-samples t-test and multiple groups by one-way ANOVA with an appropriate post-hoc test. A p-value < 0.05 was considered statistically significant.

Results

Formulation optimization

Five PLGA–DOX batches were compared on the predefined critical quality attributes (Table 1), and all fell within the target ranges. F4 gave the most balanced profile, with a diameter of 158.7 ± 3.9 nm, a PDI of 0.206 ± 0.011, a zeta potential of –28.9 ± 1.3 mV, an EE of 92.6 ± 1.4%, a DL of 13.4 ± 0.6%, and a 24-h release of 71.2 ± 1.8%. F5 produced the smallest particles and the highest loading, but its higher PDI and lower EE made it less suitable. F4 was therefore selected for all subsequent work.

Table 1. Formulation optimization based on critical quality attributes.

Batch	Size (nm)	PDI	Zeta (mV)	EE (%)	DL (%)	24-h release (%)
F1	184.6 ± 5.1	0.276 ± 0.014	–18.7 ± 1.2	82.4 ± 1.6	8.3 ± 0.4	77.6 ± 2.1
F2	176.8 ± 4.6	0.254 ± 0.013	–22.4 ± 1.1	85.7 ± 1.5	9.8 ± 0.5	75.2 ± 2.0
F3	168.2 ± 4.1	0.231 ± 0.012	–25.6 ± 1.3	89.1 ± 1.3	11.2 ± 0.5	72.8 ± 1.9
F4*	158.7 ± 3.9	0.206 ± 0.011	–28.9 ± 1.3	92.6 ± 1.4	13.4 ± 0.6	71.2 ± 1.8
F5	147.3 ± 4.8	0.274 ± 0.016	–31.6 ± 1.4	88.2 ± 1.7	14.5 ± 0.7	65.8 ± 2.2

Values are mean ± SD (n = 3). *Selected as the optimized formulation. EE,

encapsulation efficiency; DL, drug loading; PDI, polydispersity index.

Physicochemical characterization

DLS showed a unimodal population centered at 158.7 nm with a PDI of 0.206, indicating a narrow size distribution (Figure 2). The zeta potential was -28.9 mV (Figure 3), consistent with satisfactory electrostatic stabilization. The TEM-equivalent diameter (151.3 ± 8.9 nm) was slightly smaller than the hydrodynamic diameter, as expected because DLS includes the hydration layer. The particles were predominantly spherical with limited aggregation (Table 2).

Table 2. Physicochemical characteristics of the optimized F4 formulation.

Parameter	Result	Interpretation
Hydrodynamic diameter (DLS)	158.7 ± 3.9 nm	Nanoscale size suitable for cellular uptake
Polydispersity index	0.206 ± 0.011	Relatively narrow distribution
Zeta potential	-28.9 ± 1.3 mV	Satisfactory colloidal stability
TEM-equivalent diameter	151.3 ± 8.9 nm	Consistent with DLS after allowing for hydration
Morphology	Spherical / near-spherical	Limited aggregation

Values are mean ± SD (n = 3) where replicate measurements apply.

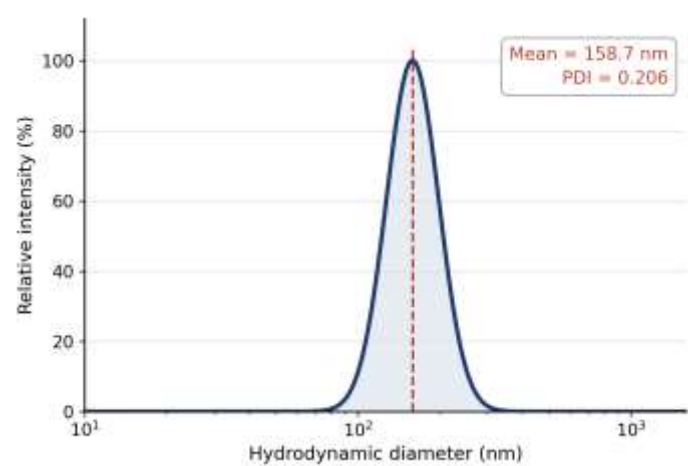


Figure 2. Particle-size distribution of the optimized nanoparticles, centered at 158.7 nm (PDI = 0.206). The curve is plotted from the reported mean and PDI.

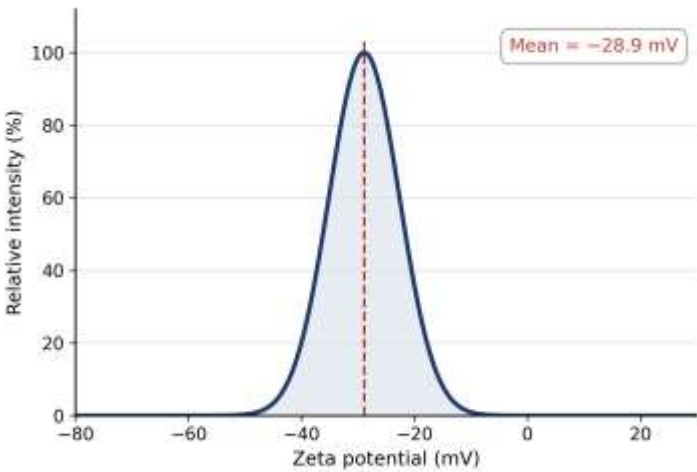


Figure 3. Zeta-potential distribution of the optimized nanoparticles (mean = -28.9 mV). The curve is plotted from the reported mean.

FT-IR, XRD, and morphology

The nanoparticle FT-IR spectrum retained the PLGA ester-carbonyl band near 1750 cm^{-1} . DOX-associated bands were attenuated and slightly shifted, and no new dominant band appeared, consistent with physical incorporation rather than covalent conjugation (Figure 4). XRD showed multiple sharp reflections for free DOX, whereas the nanoparticles showed a broad polymer halo with strong suppression of the drug peaks, indicating reduced apparent drug crystallinity after encapsulation (Figure 5). Figure 6 depicts the spherical, minimally aggregated morphology schematically.

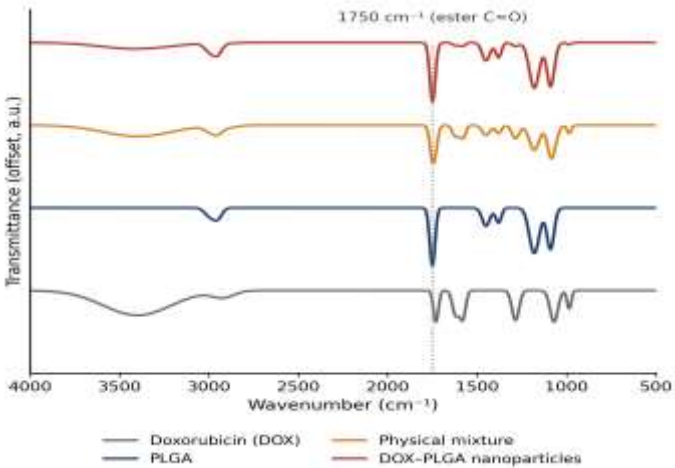


Figure 4. FT-IR spectra of doxorubicin, PLGA, their physical mixture, and DOX-PLGA nanoparticles (offset for clarity). The dotted line marks the PLGA ester-carbonyl band at 1750 cm^{-1} . Schematic reconstruction of the reported spectral features.

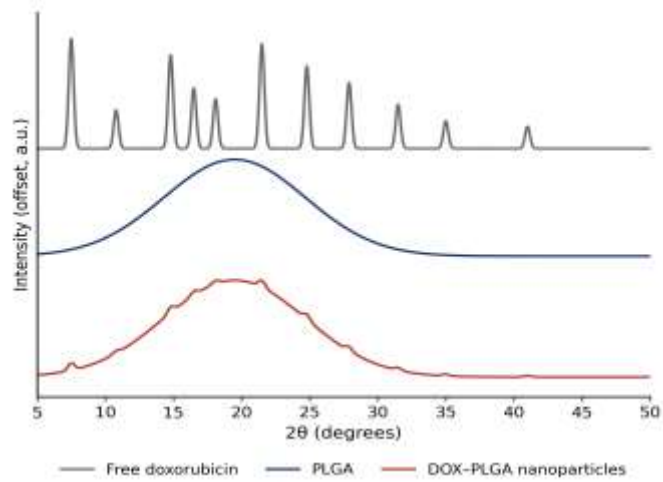


Figure 5. XRD patterns of free doxorubicin, PLGA, and DOX-PLGA nanoparticles (offset for clarity), showing loss of sharp drug reflections after encapsulation. Schematic reconstruction of the reported pattern features.

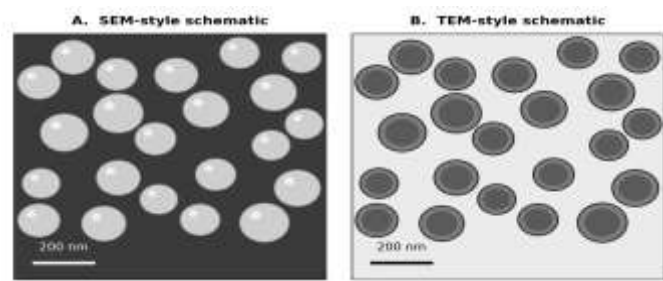


Figure 6. Schematic SEM-style (A) and TEM-style (B) renderings of the optimized nanoparticles, illustrating the reported spherical morphology.

Encapsulation and drug loading

Of the 10.0 mg of DOX input, 9.26 ± 0.14 mg was associated with the recovered nanoparticles ($EE = 92.6 \pm 1.4\%$). With a recovered nanoparticle mass of 69.1 ± 1.7 mg, DL was $13.4 \pm 0.6\%$ (Table 3). Both values lie in the upper portion of the target ranges (EE 82–94%; DL 8–15%).

Table 3. Encapsulation efficiency and drug loading of the optimized formulation.

Measurement	Mean $\pm$ SD	Calculated outcome
Initial doxorubicin input	10.00 mg	–
Encapsulated doxorubicin	9.26 ± 0.14 mg	$EE = 92.6 \pm 1.4\%$
Recovered nanoparticle mass	69.1 ± 1.7 mg	$DL = 13.4 \pm 0.6\%$

EE, encapsulation efficiency; DL, drug loading. Values are mean $\pm$ SD (n = 3).

In vitro release and kinetics

Free DOX released $67.2 \pm 1.8\%$ by 2 h and $99.1 \pm 0.7\%$ by 24 h. The nanoparticles released $23.8 \pm 1.1\%$ at 2 h, $57.9 \pm 1.6\%$ at 12 h, and $71.2 \pm 1.8\%$ at 24 h (Table 4; Figure 7). The difference was greatest during the first 8 h, showing attenuation of the initial burst and sustained release.

Table 4. Cumulative *in vitro* release of free and nanoparticle-associated doxorubicin.

Time (h)	Free drug (%)	DOX-PLGA NPs (%)
0	0.0 ± 0.0	0.0 ± 0.0
0.5	31.4 ± 1.5	9.8 ± 0.8
1	49.8 ± 1.8	15.9 ± 1.0
2	67.2 ± 1.8	23.8 ± 1.1
4	82.5 ± 1.7	34.7 ± 1.3
6	89.6 ± 1.5	42.3 ± 1.4
8	93.4 ± 1.3	48.7 ± 1.5
12	96.7 ± 1.1	57.9 ± 1.6
18	98.3 ± 0.9	65.6 ± 1.7
24	99.1 ± 0.7	71.2 ± 1.8

Values are mean $\pm$ SD (n = 3). NPs, nanoparticles.

Discussion

This study asked whether a biodegradable PLGA carrier could improve the delivery profile and *in vitro* performance of doxorubicin. The optimized F4 formulation combined a nanoscale diameter (158.7 nm), a narrow size distribution (PDI 0.206), a negative surface charge (-28.9 mV), high encapsulation (92.6%), and substantial loading (13.4%). These properties were followed by sustained release, enhanced uptake, greater target-cell inhibition, lower nonspecific toxicity, and a 1.8-fold gain in apparent potency. This coherent pattern supports the central hypothesis that nanoformulation improves the balance between delivery efficiency and therapeutic activity.F4 was selected for its balance across several critical quality attributes rather than for any single value, in keeping with the quality-attribute framework recommended for nanomedicines (Taha *et al.*, 2020). A diameter near 159 nm and a PDI near 0.21 indicate a nanoscale and reasonably uniform population, which is relevant to colloidal behavior, tissue transport, internalization, and reproducibility of exposure (Mitchell *et al.*, 2021; Öztürk *et al.*, 2024). The smaller TEM-

equivalent size is physically plausible, because DLS measures the hydrated particle whereas microscopy reflects the dry core (Filippov *et al.*, 2023). The zeta potential of -28.9 mV suggests a meaningful electrostatic contribution to stability. However, zeta potential alone cannot establish storage or *in vivo* stability, because ionic strength and protein-corona formation in biological fluids can change the effective surface properties (Nienhaus & Nienhaus, 2023). Retention of the PLGA carbonyl band, together with attenuated and shifted DOX bands and no new dominant absorption, supports physical drug-polymer association rather than covalent modification. Suppression of the sharp DOX reflections in XRD suggests reduced crystallinity, consistent with molecular dispersion or an amorphous fraction within the matrix, which may influence dissolution and diffusion. Neither technique proves quantitative encapsulation, which was established by direct drug-content measurement. A spherical, minimally aggregated morphology is typical of emulsion-solvent evaporation products, but it depends on preparation and imaging conditions. Final confirmation should therefore rely on several representative, instrument-acquired micrographs with documented image selection. High EE and DL indicate that most of the drug was retained while a useful payload was delivered per unit of carrier, which reduces the carrier burden needed for a given dose. The release profile was the clearest functional distinction from the free drug: near-complete release within 24 h for free DOX versus 71.2% for F4, with a much smaller early burst. This is consistent with PLGA acting as a diffusion- and degradation-controlled matrix (Kopeček & Yang, 2020; Beach *et al.*, 2024). A Korsmeyer-Peppas exponent of 0.556 indicates anomalous transport in which diffusion is accompanied by polymer relaxation, hydration, or erosion. This mechanistic reading is plausible for PLGA but is not unique proof, because model fits depend on the sampling schedule, sink conditions, and the fraction of the curve used. The best docking score (-8.7 kcal/mol) and the plausible hydrogen-bonding, stacking, and hydrophobic contacts agree with the established molecular action of doxorubicin and provide a computational link between delivery and target engagement. Docking does not show that encapsulation strengthens binding. It supports the plausibility that drug released after internalization retains target-compatible interactions. This component should remain supportive until it is reproduced with a documented structure, grid definition, validation ligand, and scoring protocol (Bao *et al.*, 2023). The 57.0% rise in intracellular signal is consistent with endocytic entry of nanoscale carriers and altered intracellular trafficking of the payload (Cong *et al.*, 2022). Fluorescence reports drug-associated signal, not necessarily internalized or released drug, so confocal imaging,

flow-cytometric controls, and endosomal colocalization are needed to distinguish surface-bound, internalized, and released fractions. Greater uptake was accompanied by higher target-cell inhibition (60.0% to 79.2%) and a lower IC₅₀ (3.6 to 2.0 $\mu\text{g/mL}$), while nonspecific toxicity fell from 30.0% to 21.6%. Enhancing activity without a proportional increase in injury to healthy cells is the practical goal of cancer nanomedicine (Peer *et al.*, 2007; Shi *et al.*, 2017; Bhatia *et al.*, 2022). These data do not, however, establish active targeting. The formulation carries no receptor-specific ligand, so the improvement should be described as formulation-mediated enhancement of delivery and uptake. True targeting requires evidence that the carrier reaches or enters the intended cells through a defined mechanism and retains that selectivity in complex biological settings (Dilliard & Siegwart, 2023; Veiga *et al.*, 2023). Taken together, uniform particles with high drug incorporation released the drug more slowly, delivered more drug-associated signal to cells, and produced stronger target-cell inhibition with less nonspecific toxicity. The 1.8-fold potency gain thus arises from a chain of mutually supportive observations rather than from one isolated measurement, in line with the multistage nature of nanoparticle performance (Blanco *et al.*, 2015; Veiga *et al.*, 2023). An *in vitro* potency gain, however, cannot be assumed to translate *in vivo*, where protein adsorption, immune recognition, vascular transport, clearance, and tumor heterogeneity can reduce benefit. The results are proof-of-concept evidence that justifies more rigorous testing, not evidence of clinical superiority. Several limitations should be considered when interpreting these findings. All biological evidence comes from cell models. Pharmacokinetics, biodistribution, immune interactions, efficacy, and cardiac safety, which are central to doxorubicin therapy, were not assessed. The nanoparticles carry no receptor-specific ligand. The improvements in uptake and selectivity reflect formulation-mediated delivery and must not be interpreted as receptor-mediated targeting. Experiments used $n = 3$, which limits statistical power and the precision of fitted parameters such as the IC₅₀ and the release exponent. Primary replicate-level data and independent batch-to-batch reproducibility should be reported. Release was followed for 24 h in a single dialysis setup. Longer observation, additional pH values, and media that approximate extracellular and intracellular environments are needed, and the kinetic models were compared on a limited number of time points. Fluorescence-based quantification reflects drug-associated signal and cannot distinguish surface-bound, internalized, and released drug. Docking is a single-target, single-protocol analysis, and the morphology panels and interaction map are schematics rather than instrument-acquired images. FT-IR, XRD, and light-scattering graphics

should be replaced or confirmed with original instrument output. Colloidal and drug-content stability during storage, and behavior in serum-containing media where a protein corona forms, were not evaluated. One cancer-cell model and one nonmalignant cell model were used, so the generality of the selectivity and potency results across tumor types and normal tissues is unknown.

Conclusion

Doxorubicin-loaded PLGA nanoparticles prepared by emulsion-solvent evaporation and optimized across five batches yielded a lead formulation (F4) with a hydrodynamic diameter of 158.7 nm, a PDI of 0.206, a zeta potential of -28.9 mV, an encapsulation efficiency of 92.6%, and a drug loading of 13.4%. Spectroscopic and diffraction analyses indicated physical incorporation of the drug with reduced crystallinity. Release was sustained to 71.2% at 24 h and followed anomalous transport (Korsmeyer-Peppas n = 0.556). Compared with the free drug, the nanoparticles increased cellular uptake by 57.0%, raised target-cell inhibition from 60.0% to 79.2%, lowered nonspecific toxicity by 28.0%, and improved the apparent IC50 from 3.6 to 2.0 µg/mL. Docking offered supportive evidence of target-compatible interactions. These results establish the formulation as a proof-of-concept platform for further preclinical development. Because the study is limited to *in vitro* models and the carrier lacks a targeting ligand, active targeting and *in vivo* benefit remain to be demonstrated through ligand functionalization, stability testing, and pharmacokinetic, biodistribution, efficacy, and safety studies.

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

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Authors’ Contributions: All authors contributed substantially to the conception and design of the study, data acquisition, analysis, or interpretation. All authors participated in drafting or critically revising the manuscript, approved the final version, and agreed to be accountable for all aspects of the work in accordance with ICMJE recommendations.

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