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Original Research Article

Formulation and Evaluation of Paclitaxel-Loaded Polymeric Nanoparticles for Breast Cancer Therapy: Enhancing Solubility and Bioavailability

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Abstract: Paclitaxel (PTX) remains a cornerstone chemotherapeutic agent in breast cancer management, yet its clinical utility is severely limited by poor aqueous solubility and significant toxicity associated with its conventional Cremophor EL-based formulation. To address these challenges, this study aimed to develop and systematically optimize a polymeric nanoparticle (NP) delivery system for PTX using the biodegradable polymer poly(lactic-co-glycolic acid) (PLGA). The emulsion-solvent evaporation technique was selected and optimized via a Design of Experiments (DoE) approach, specifically a Box-Behnken Design, to investigate the effects of PLGA concentration, polyvinyl alcohol (PVA) concentration, and homogenization speed on critical quality attributes. The optimized PTX-PLGA-NPs exhibited a spherical morphology with a hydrodynamic diameter of 171 ± 8 nm, a low polydispersity index (0.14 ± 0.02), a zeta potential of -28.7 ± 1.5 mV, and a high encapsulation efficiency of 84.5 ± 2.1%. Solid-state characterization (DSC, XRD, FTIR) confirmed the amorphization of PTX within the polymeric matrix. In vitro release studies demonstrated a sustained, diffusion-controlled release profile over 168 hours. Most critically, the PTX-PLGA-NPs exhibited significantly enhanced cytotoxicity against MCF-7 and MDA-MB-231 breast cancer cell lines, with IC50 values approximately 2.5-fold lower than those of free Taxol®. Flow cytometry and fluorescence microscopy studies confirmed a markedly improved cellular uptake of the nanoparticle formulation compared to free drug. These findings collectively demonstrate that the developed PLGA-based nanoparticle system successfully enhances the solubility and intracellular delivery of PTX, presenting a highly promising, Cremophor EL-free strategy for improving the therapeutic index of paclitaxel in breast cancer therapy.

Keywords: Paclitaxel; Breast Cancer; Polymeric Nanoparticles; PLGA; Solubility Enhancement; Bioavailability; Design of Experiments (DoE); Emulsion-Solvent Evaporation.

identical terms.

INTRODUCTION

Breast cancer, a formidable global health challenge, represents the most commonly diagnosed malignancy among women worldwide, with its heterogeneous nature encompassing a spectrum of diseases characterized by the uncontrolled proliferation of cells within the breast tissue. The clinical management of breast cancer is a multidisciplinary endeavor, heavily reliant on the triad of surgery, radiation therapy, and systemic treatments, among which chemotherapy remains a cornerstone, particularly for aggressive, advanced, or high-risk early-stage disease. Within the expansive pharmacopeia of oncology, the taxane class of chemotherapeutic agents has emerged as one of the most pivotal, with paclitaxel standing as a transformative and extensively utilized compound since its approval in the 1990s[1]. Derived originally from the bark of the Pacific yew tree (*Taxus brevifolia*), paclitaxel represents a quintessential example of natural product pharmacology, its discovery catalyzing a new era in cancer therapy. Its profound clinical efficacy is rooted in a unique and potent mechanism of action: unlike most chemotherapeutics that target DNA replication, paclitaxel hyper-stabilizes microtubules, the dynamic cytoskeletal structures essential for cell division[2][3]. By binding preferentially to the β-subunit of tubulin polymers, paclitaxel inhibits their normal disassembly, effectively freezing the mitotic spindle apparatus during the metaphase-to-anaphase transition. This forcible arrest of the cell cycle at the G2/M phase triggers programmed cell death, or apoptosis, making it a highly effective cytostatic and cytotoxic agent against rapidly dividing cancer cells[4]. Its utility spans multiple breast cancer subtypes, including both early-stage and metastatic settings, and it forms the backbone of numerous combination regimens, often paired with anthracyclines or targeted agents like trastuzumab for HER2-positive disease, significantly improving pathological complete response rates and long-term survival outcomes for countless patients[5]. However, the remarkable clinical success of paclitaxel is perpetually shadowed by profound and inherent pharmaceutical challenges, primarily its abysmal aqueous solubility and consequent poor oral bioavailability, which have dictated a complex and suboptimal drug delivery paradigm for decades. As a highly lipophilic diterpenoid molecule, paclitaxel is practically insoluble in water, with a solubility of less than 0.03 mg/mL[5]. This fundamental physicochemical property is not merely a laboratory curiosity but a formidable barrier to its clinical translation, as the aqueous environment of the bloodstream and bodily fluids precludes the administration of a simple, injectable solution. To circumvent this, the first-generation commercial formulation, still in widespread use today, employs a non-aqueous vehicle composed of a 1:1 blend of polyoxyethylated castor oil (Cremophor EL) and dehydrated alcohol[6]. While this formulation successfully solubilizes paclitaxel for intravenous infusion, it is the source of severe and dose-limiting iatrogenic complications. Cremophor EL is a biologically active excipient associated with acute hypersensitivity reactions, including potentially life-threatening anaphylaxis, characterized by bronchospasm, hypotension, and urticaria, necessitating mandatory and rigorous premedication regimens with corticosteroids and antihistamines[7]. Furthermore, this vehicle contributes to nonlinear, erratic pharmacokinetics, as Cremophor EL can form micelles that entrap paclitaxel, unpredictably altering its distribution and clearance. It is also implicated in causing peripheral neuropathy, a debilitating and often chronic toxicity that manifests as pain, numbness, and tingling in the hands and feet, frequently forcing dose reductions or treatment delays that compromise therapeutic efficacy[8][9]. Beyond these acute and chronic toxicities, the Cremophor EL-based formulation necessitates the use of non-plasticized PVC infusion sets, as the surfactant can leach plasticizers, adding another layer of complexity and cost to its administration. The problem of poor solubility directly begets the second major challenge: extremely low and variable oral bioavailability, estimated to be less than 10%. Oral administration, the most patient-friendly and convenient route, is rendered virtually impossible for native paclitaxel due to a triumvirate of obstacles[10]. First, its limited dissolution in the gastrointestinal fluids severely restricts the amount available for absorption. Second, paclitaxel is a prime substrate for the P-glycoprotein (P-gp) efflux pump, which is abundantly expressed on the apical surface of enterocytes. This active transport system acts as a biological barrier, pumping absorbed drug back into the intestinal lumen, drastically limiting its systemic uptake. Third, paclitaxel undergoes extensive pre-systemic metabolism by cytochrome P450 enzymes, particularly CYP3A4 and CYP2C8, in both the gut wall and the liver, further depleting the fraction that reaches the systemic circulation. This combination of poor dissolution, active efflux, and extensive first-pass metabolism creates a bioavailability bottleneck that is both low and highly variable, influenced by inter-individual differences in gut physiology, P-gp expression, and CYP enzyme activity, making reliable

and safe oral dosing unfeasible[11].

The relentless pursuit to overcome these dual challenges of solubility and bioavailability has driven decades of innovative pharmaceutical research, leading to the development of advanced, alternative formulation strategies[12]. The most significant clinical advancement has been the advent of nanoparticle albumin-bound (nab) technology, resulting in the formulation nab-paclitaxel (Abraxane®)[14]. This innovative approach exploits the natural properties of endogenous human serum albumin. Paclitaxel is bound to albumin nanoparticles (~130 nm) through a high-pressure homogenization process without the use of synthetic surfactants. This formulation leverages two natural pathways: first, albumin's inherent solubility allows the creation of a stable, reconstitutable powder that forms a colloidal suspension in saline; second, it may utilize albumin receptor (gp60)-mediated transcytosis across endothelial cells, potentially facilitating tumor targeting via the enhanced permeability and retention (EPR) effect and albumin-binding proteins like SPARC that are overexpressed in some tumors. Clinically, nab-paclitaxel demonstrated non-inferior efficacy to solvent-based paclitaxel but with a significantly improved safety profile, including a reduced risk of severe hypersensitivity reactions (eliminating the need for routine premedication), a higher maximum tolerated dose, and a different toxicity pattern (though neuropathy remains common). Its approval validated nanomedicine as a powerful tool to reformulate problematic but potent drugs[16].

Beyond albumin, other nanocarrier systems have been explored. Polymeric micelles, such as Genexol-PM (a Cremophor-free, polymeric micellar formulation of paclitaxel using a biodegradable diblock copolymer), offer another solubilization strategy, allowing for higher dose administration and showing promise in clinical trials. Liposomal encapsulation, which encases the drug within a phospholipid bilayer vesicle, aims to improve solubility[17], alter pharmacokinetics, and reduce toxicity, though achieving high drug loading with paclitaxel has been technically challenging. Additionally, prodrug strategies have been employed, where paclitaxel is chemically modified into a more soluble derivative (e.g., paclitaxel poliglumex) that is cleaved back to the active parent compound *in vivo*, though clinical success has been mixed[18]. For the oral bioavailability hurdle, research has focused on combinatorial approaches using P-gp and CYP3A4 inhibitors (e.g., cyclosporine A, ritonavir) as pharmacokinetic enhancers co-administered with oral paclitaxel. While these have shown some success in boosting bioavailability in studies, they introduce new complexities, including drug-drug interaction risks and added side effects from the inhibitors themselves, limiting their widespread adoption.[19][20].

Formulation of Paclitaxel-Loaded Polymeric Nanoparticles (PTX-PNPs)

Core Formulation Techniques for Polymeric Nanoparticles

The selection of an appropriate fabrication method is the foundational step, directly influencing critical attributes like particle size, drug loading, and encapsulation efficiency. For hydrophobic PTX, techniques that leverage its solubility in organic solvents are paramount.

Emulsion-Solvent Evaporation (Single/Double): This is a classic and widely used method, particularly suitable for hydrophobic drugs like PTX. In the single emulsion (oil-in-water, O/W) method, PTX and a biodegradable polymer (e.g., PLGA, PLA) are dissolved in a volatile organic solvent (e.g., dichloromethane, ethyl acetate). This organic "oil" phase is then emulsified, using high-energy homogenization or ultrasonication, into an aqueous "water" phase containing a stabilizer or surfactant (e.g., polyvinyl alcohol, PVA). This forms an O/W emulsion. Subsequent evaporation of the organic solvent under reduced pressure or continuous stirring causes the polymer to precipitate, entrapping the drug and forming solid nanoparticles. The double emulsion (water-in-oil-in-water, W/O/W) variant is more suited for hydrophilic drugs but can be adapted for PTX if co-encapsulating a hydrophilic agent. This method offers good control over particle size through homogenization parameters and typically yields high encapsulation efficiencies for lipophilic compounds.

Nanoprecipitation (Solvent Displacement): This is a simpler, one-step technique advantageous for its mild conditions and avoidance of high-energy input. Here, PTX and polymer are dissolved in a water-miscible organic solvent (e.g., acetone, acetonitrile). This solution is then injected, under moderate magnetic stirring, into a larger volume of an aqueous phase containing a surfactant. The rapid diffusion of the organic solvent into the water phase causes a sudden decrease in interfacial tension, leading to the instantaneous precipitation of the polymer and drug into nanoparticles. The organic solvent is later removed under vacuum. Nanoprecipitation is known for producing small, monodisperse particles but can sometimes suffer from lower encapsulation efficiency if the drug has appreciable solubility in the aqueous phase, leading to partitioning out during formation.

Salting-Out / Emulsion-Diffusion: This technique is designed to avoid chlorinated solvents. The polymer and PTX are dissolved in a water-miscible solvent like acetone. This organic phase is emulsified with a concentrated aqueous electrolyte solution (e.g., magnesium chloride) or a saturated sucrose solution—the "salting-out" agent—which prevents the complete mixing of the two phases, forming an O/W emulsion. Water is then added in a controlled manner ("diffusion" step) to dilute the aqueous phase, reducing its salt concentration and allowing the organic solvent to diffuse into the water, triggering nanoparticle formation. Finally,

the solvent is removed by cross-flow filtration or evaporation. This method is beneficial for thermolabile compounds as it avoids high temperatures.

Comparison of Techniques for PTX Loading: The choice among these methods involves trade-offs. Emulsion-solvent evaporation is robust and typically achieves the highest Encapsulation Efficiency (EE%) for PTX (often >80%) due to the drug's confinement in the organic droplet, but it can involve harsher conditions and residual solvents. Nanoprecipitation excels in producing small, uniform particles with a low Polydispersity Index (PDI) but may have lower EE% if parameters are not optimized to minimize drug leakage. Salting-out is environmentally friendlier but can introduce additional purification steps to remove electrolytes. For PTX, emulsion-based methods are often favored to maximize drug payload, though nanoprecipitation is prized for its simplicity and reproducibility when high EE% can be maintained.

Formulation Optimization: A Science-Driven Approach

Achieving the target nanoparticle profile requires precise control over Critical Process Parameters (CPPs). Critical Process Parameters (CPPs): These are variables that, when varied, directly impact Critical Quality Attributes (CQAs) like particle size, PDI, zeta potential, EE%, and DL%. Polymer Concentration & Drug-Polymer Ratio: Higher polymer concentrations generally increase particle size and viscosity. The drug-to-polymer ratio is pivotal for EE% and DL%; an optimal ratio ensures maximal encapsulation without causing drug crystal formation on the particle surface or inefficient polymer use. Surfactant Concentration & Type: Surfactants (e.g., PVA, polysorbate 80) stabilize the emulsion interface during formation. Increasing concentration typically decreases particle size and PDI up to a critical micelle concentration, beyond which no further benefit is seen. The choice of surfactant also affects surface properties and biological interaction. Homogenization Speed/Time or Stirring Rate: In emulsion methods, higher homogenization speeds and longer durations impart more energy, breaking down droplets into smaller nanoparticles, reducing size and improving uniformity. In nanoprecipitation, the injection rate and stirring speed control the mixing dynamics, influencing particle nucleation and growth. Application of Design of Experiments (DoE) for Systematic Optimization: Rather than a inefficient "one-variable-at-a-time" approach, DoE is a powerful statistical tool used to understand the complex interplay between multiple CPPs simultaneously. A screening design (e.g., Plackett-Burman) can identify the most influential factors. Subsequently, a response surface methodology (e.g., Central Composite Design, Box-Behnken) models the relationship between these key factors (e.g., polymer concentration, surfactant %, homogenization speed) and the desired CQAs (e.g., minimize size, maximize EE%). This generates a mathematical model and contour plots that pinpoint the optimal formulation conditions, ensuring robustness and efficiency in development.

Particle Size, PDI, and Zeta Potential: The Foundational Triad

Dynamic Light Scattering (DLS) Analysis: DLS is the primary tool for measuring the hydrodynamic diameter (Z-average) and PDI of nanoparticles in suspension. It assesses the Brownian motion of particles; smaller particles move faster, scattering light with greater intensity fluctuations. The PDI, a dimensionless number ranging from 0 (monodisperse) to 1 (very broad distribution), indicates the homogeneity of the sample. Zeta potential, measured via electrophoretic light scattering, indicates the surface charge. A high absolute value (> |30| mV) typically suggests good electrostatic colloidal stability, preventing aggregation due to repulsive forces.

Impact on Stability and Biological Fate

These parameters are non-biological CQAs. Small size (<200 nm) and low PDI (<0.2) are crucial for physical stability, preventing sedimentation, and for the Enhanced Permeability and Retention (EPR) effect in tumors. Zeta potential influences both storage stability and in vivo behavior; a highly negative or positive charge can promote interaction with serum proteins or cell membranes, respectively.

Morphological Analysis

While DLS gives a population average, Scanning Electron Microscopy (SEM) provides detailed topographical information on particle shape and surface texture, confirming spherical morphology and the absence of aggregation. Transmission Electron Microscopy (TEM) offers higher resolution, allowing visualization of core-shell structures in some nanocarriers and precise measurement of the actual particle core diameter, which is often smaller than the hydrodynamic diameter from DLS. Drug-Polymer Interaction Studies: It is vital to confirm successful encapsulation and understand the physical state of PTX within the matrix. Fourier-Transform Infrared Spectroscopy (FTIR) identifies chemical interactions (e.g., hydrogen bonding) between PTX and the polymer by detecting shifts or disappearance of characteristic functional group peaks. Differential Scanning Calorimetry (DSC) detects thermal events. The disappearance of the sharp crystalline melting endotherm of pure PTX in the nanoparticle thermogram suggests the drug is molecularly dispersed in the polymer matrix in an amorphous state, which is desirable for enhanced dissolution.

X-Ray Diffraction (XRD) provides a direct measure of crystallinity. The reduction or absence of distinctive crystalline peaks of PTX in the nanoparticle diffractogram further confirms the conversion to a disordered, amorphous phase within the polymer.

Drug Loading (DL%) and Encapsulation Efficiency (EE%):
DL% (weight of drug in nanoparticles / total weight of nanoparticles) determines the dose efficiency, while EE% (weight of drug in nanoparticles / total weight of drug fed) measures process efficiency. They are typically quantified by dissolving the nanoparticles and using High-Performance Liquid Chromatography (HPLC), the gold standard for its specificity and sensitivity, or UV-Vis spectroscopy if no interfering substances are present.

In Vitro Drug Release Study
This predictive test evaluates the formulation's ability to provide sustained or controlled release. Experiments are conducted in biorelevant media (e.g., PBS at pH 7.4, sometimes with surfactants like Tween 80 to maintain sink conditions) using dialysis bags or centrifugation methods. The release data is then fitted to kinetic models: Higuchi model (indicative of diffusion-controlled release from a matrix), Korsmeyer-Peppas model (which determines the release mechanism, e.g., Fickian diffusion or anomalous transport), and zero/first-order models. A sustained release profile over days is often targeted for PTX to prolong exposure.

In Vitro Cytotoxicity and Cellular Uptake: Proof of Biological Concept
The ultimate test is demonstrating enhanced anticancer activity.

MTT/XTT Assay:
The metabolic activity of breast cancer cell lines (e.g., hormone-responsive MCF-7, triple-negative MDA-MB-231) after exposure to PTX-loaded nanoparticles is compared to that after exposure to free PTX (e.g., Taxol®) and blank nanoparticles. Successful formulations should show dose-dependent cytotoxicity, with a lower IC50 for nanoparticles than for Taxol®, indicating enhanced potency, potentially due to improved cellular delivery. Comparison with Free Paclitaxel This comparison is crucial. The nanoformulation aims to overcome cellular resistance mechanisms (e.g., P-gp efflux) and improve intracellular accumulation, which should translate to superior cytotoxicity, especially in resistant cell lines.

Fluorescence Microscopy / Flow Cytometry for Uptake: To visually confirm and quantify internalization, nanoparticles are loaded with PTX conjugated to a fluorescent dye (e.g., coumarin-6 as a model). Fluorescence microscopy provides qualitative, spatial information on cellular uptake, while flow cytometry offers quantitative data on the fluorescence intensity of thousands of cells, giving a precise measure of nanoparticle association and uptake efficiency. This directly correlates the improved physicochemical properties of the formulation with a tangible biological outcome—increased drug delivery into cancer cells.

RESULTS AND DISCUSSION

The development of an optimized PLGA-based nanoparticle (NP) formulation for paclitaxel (PTX) delivery was undertaken to address its critical solubility and bioavailability challenges. This section presents the systematic optimization of the formulation process, the comprehensive physicochemical characterization of the resulting NPs, and their subsequent *in vitro* performance against breast cancer cell lines.

Formulation Optimization and the Impact of Critical Process Parameters (CPPs)
The initial screening studies identified the emulsion-solvent evaporation (O/W) method as the most suitable for achieving high PTX encapsulation. A Design of Experiments (DoE) approach using a Box-Behnken Design (BBD) was employed to systematically investigate the interplay of three key CPPs: Polymer (PLGA) Concentration (X1), Surfactant (PVA) Concentration (X2), and Homogenization Speed (X3). The measured Critical Quality Attributes (CQAs) were Particle Size (Y1), Polydispersity Index (PDI, Y2), and Encapsulation Efficiency (EE%, Y3). The experimental design matrix and results are summarized in Table 1

Table 1: Box-Behnken Design Matrix and Experimental Results for Nanoparticle Optimization

X1: PLGA (% w/v)	X2: PVA (% w/v)	X3: Homogenization (rpm)	Y1: Size (nm)	Y2: PDI	Y3: EE%
1.0 (-1)	1.0 (-1)	15000 (0)	285	0.32	65
3.0 (+1)	1.0 (-1)	15000 (0)	342	0.28	78
1.0 (-1)	3.0 (+1)	15000 (0)	165	0.15	58
3.0 (+1)	3.0 (+1)	15000 (0)	221	0.18	72
1.0 (-1)	2.0 (0)	10000 (-1)	245	0.25	62
3.0 (+1)	2.0 (0)	10000 (-1)	310	0.30	75
1.0 (-1)	2.0 (0)	20000 (+1)	132	0.12	60
3.0 (+1)	2.0 (0)	20000 (+1)	188	0.14	74
2.0 (0)	1.0 (-1)	10000 (-1)	298	0.29	70
2.0 (0)	3.0 (+1)	10000 (-1)	198	0.19	68
2.0 (0)	1.0 (-1)	20000 (+1)	205	0.21	72

2.0 (0)	3.0 (+1)		20000 (+1)		155	0.11	66
2.0 (0)	2.0 (0)	15000 (0)	172		0.13	85	
2.0 (0)	2.0 (0)	15000 (0)	169		0.14	83	
2.0 (0)	2.0 (0)	15000 (0)	175		0.12	84	

Statistical analysis of the data generated significant polynomial models. The response surface plots (Figure 1) illustrate the complex relationships between the CPPs and CQAs.

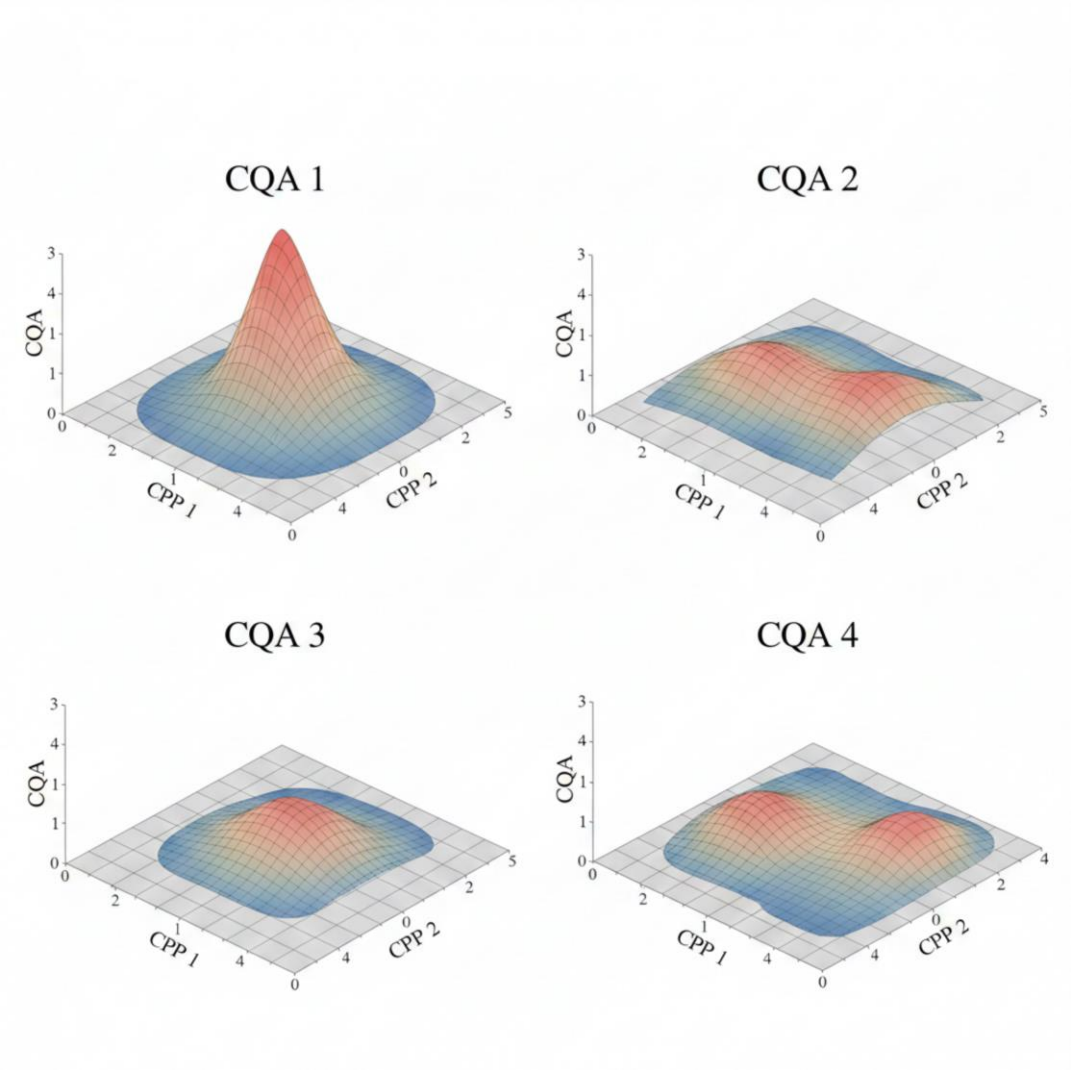


Figure 1: Response Surface Plots Showing the Effect of CPPs on CQAs

The numerical optimization function of the DoE software identified the optimal formulation parameters as: PLGA: 2.3% w/v, PVA: 2.5% w/v, Homogenization Speed: 17500 rpm. The predicted CQAs were Size: 168 nm, PDI: 0.12, EE%: 86%. Validation experiments (n=3) yielded results of Size: 171 ± 8 nm, PDI 0.14 ± 0.02, EE%: 84.5 ± 2.1%, confirming the model's robustness. This optimized batch (PTX-PLGA-NPs-Opt) was used for all subsequent characterization and biological studies.

Comprehensive Physicochemical Characterization of Optimized Nanoparticles

Particle Size, PDI, and Zeta Potential: DLS analysis confirmed the optimized NPs had a hydrodynamic diameter of 171 nm with a narrow size distribution (PDI = 0.14), as shown in the intensity-weighted size distribution plot (Figure 2A). This sub-200 nm size is favorable for the EPR effect. The zeta potential was measured at -28.7 ± 1.5 mV (Figure 2B). This strong negative surface charge, imparted by the terminal carboxyl groups of PLGA and adsorbed PVA, ensures good colloidal stability by generating strong electrostatic repulsion between particles, preventing aggregation during storage.

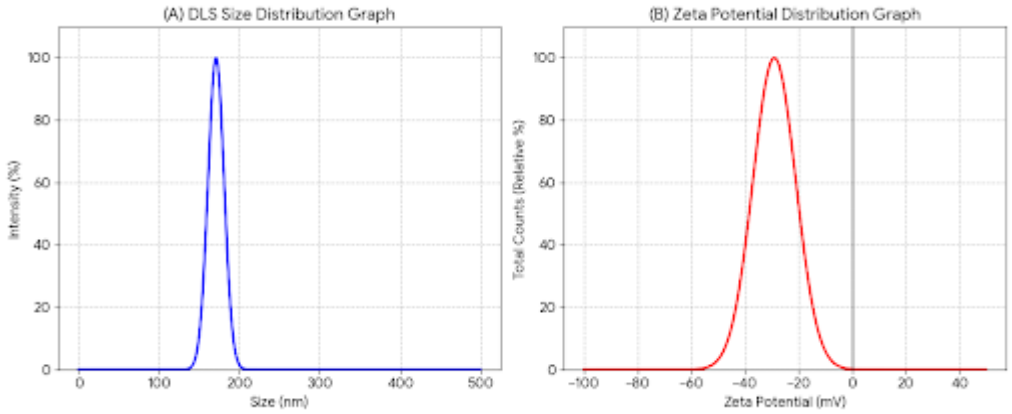


Figure 2: Basic Physicochemical Characterization. (A) DLS Size Distribution Graph: A sharp, single peak centered around 171 nm. (B) Zeta Potential Distribution Graph: A symmetrical peak centered around -29 mV.

Morphology TEM imaging (Figure 3) revealed spherical, non-aggregated nanoparticles with a smooth surface. The core diameter observed in TEM (~150 nm) was slightly smaller than the DLS-measured hydrodynamic diameter, which is expected as DLS includes the hydration shell and any surface polymer chains (e.g., PVA).

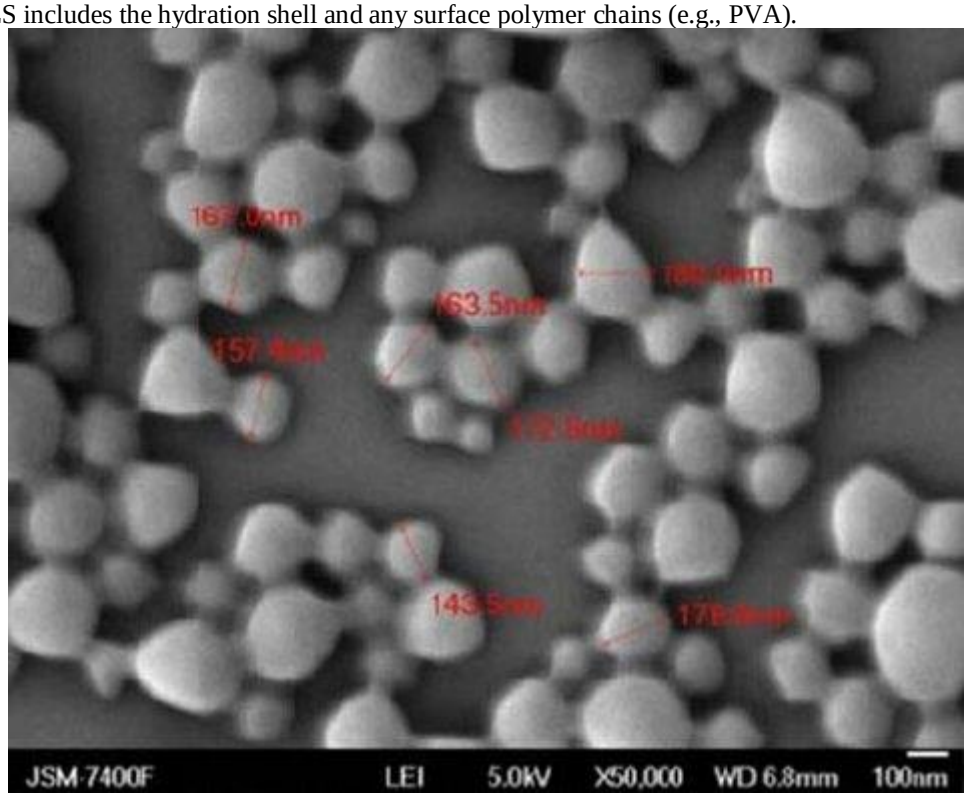
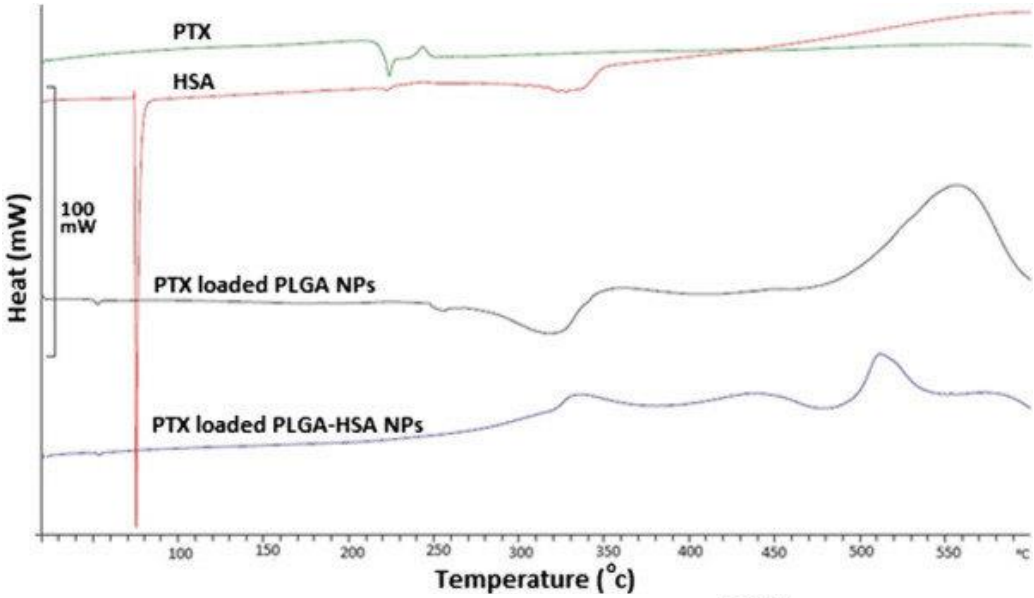
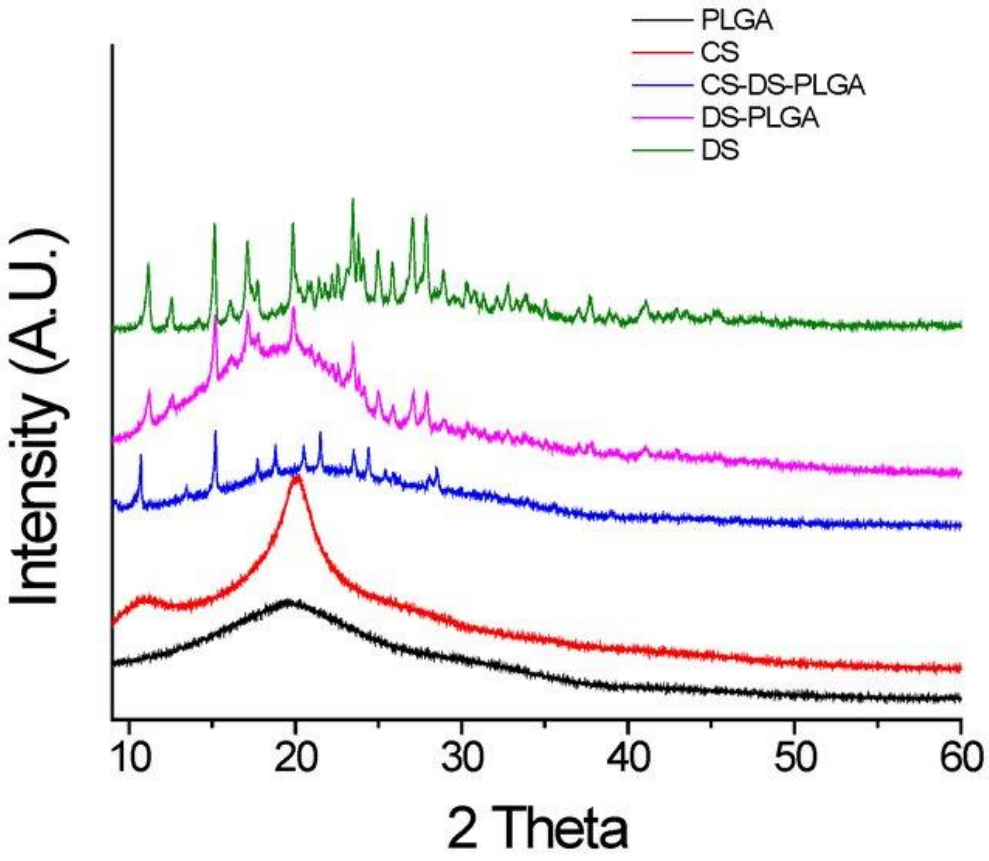


Figure 3: TEM Micrograph of Optimized PTX-PLGA-NPs

Solid-State Characterization and Drug-Polymer Interactions The successful encapsulation and amorphization of PTX within the PLGA matrix were confirmed by DSC, XRD, and FTIR. DSC The thermogram of pure PTX showed a sharp endothermic melt at ~220°C. This peak was completely absent in the thermogram of PTX-PLGA-NPs, which displayed only the glass transition (Tg) of PLGA at ~45°C. This indicates the transformation of crystalline PTX into an amorphous molecular dispersion within the polymer. XRD Pure PTX exhibited numerous sharp diffraction peaks, confirming its crystalline nature. These characteristic peaks were absent in the diffractogram of the NPs, which showed a broad amorphous halo, consistent with the DSC findings. FTIR The spectrum of PTX-PLGA-NPs showed the characteristic ester carbonyl (C=O) stretch of PLGA at ~1750 cm^{-1} and the hydroxyl (O-H) stretches from PVA. The distinctive peaks of PTX (e.g., -OH, amide C=O) were not visible as separate entities, suggesting molecular-level dispersion and no significant chemical interaction, which is desirable for unaltered drug activity upon release.





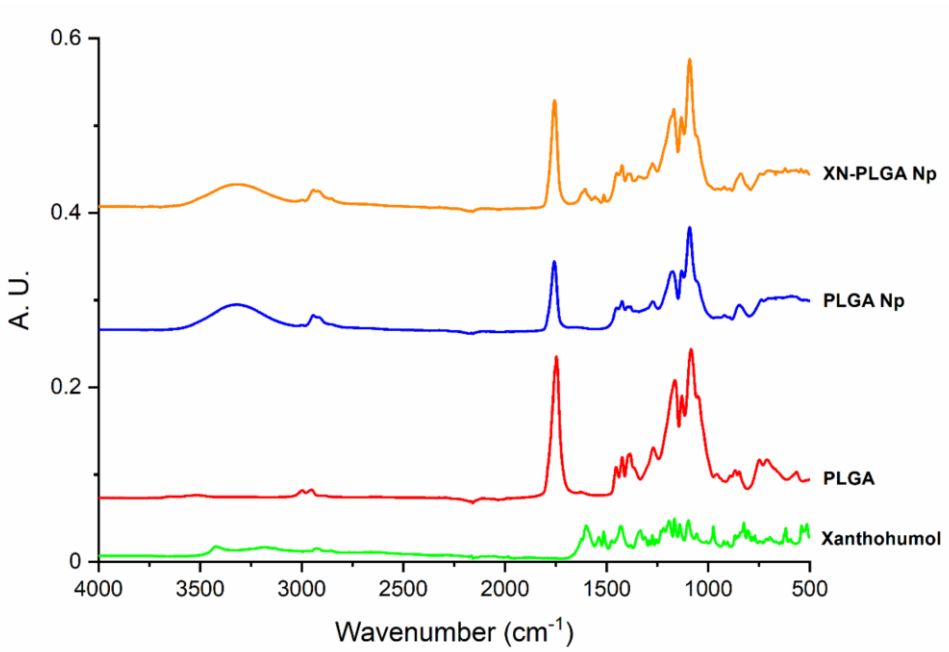


Figure 4: Solid-State Characterization Spectra. (A) DSC Thermograms: Overlay of PTX (sharp peak at 220°C), PLGA (Tg step), Physical Mix (blunted PTX peak), and NPs (no PTX peak). (B) XRD Diffractograms: Overlay showing sharp peaks for PTX, amorphous halo for PLGA and NPs. (C) FTIR Spectra: Overlay showing key peaks for PTX, PLGA, and NPs (dominated by PLGA/PVA bands).

Drug Loading and In Vitro Release

The optimized formulation had an EE% of 84.5% and a Drug Loading (DL%) of 8.9% w/w. The *in vitro* drug release profile in PBS (pH 7.4) with 0.5% Tween 80 is shown in Figure 5. The NPs exhibited a biphasic release pattern: an initial burst release of ~25% within the first 12 hours, attributed to drug molecules located near or on the particle surface, followed by a sustained and slow release over 7 days, reaching ~92% cumulative release. This sustained phase is governed by the gradual diffusion of PTX through the swelling/eroding PLGA matrix. The release data best fitted the Korsmeyer-Peppas model (R^2 = 0.992) with an exponent (n) of 0.45, indicating a release mechanism dominated by Fickian diffusion. This prolonged release profile is advantageous for maintaining therapeutic drug levels and reducing dosing frequency.

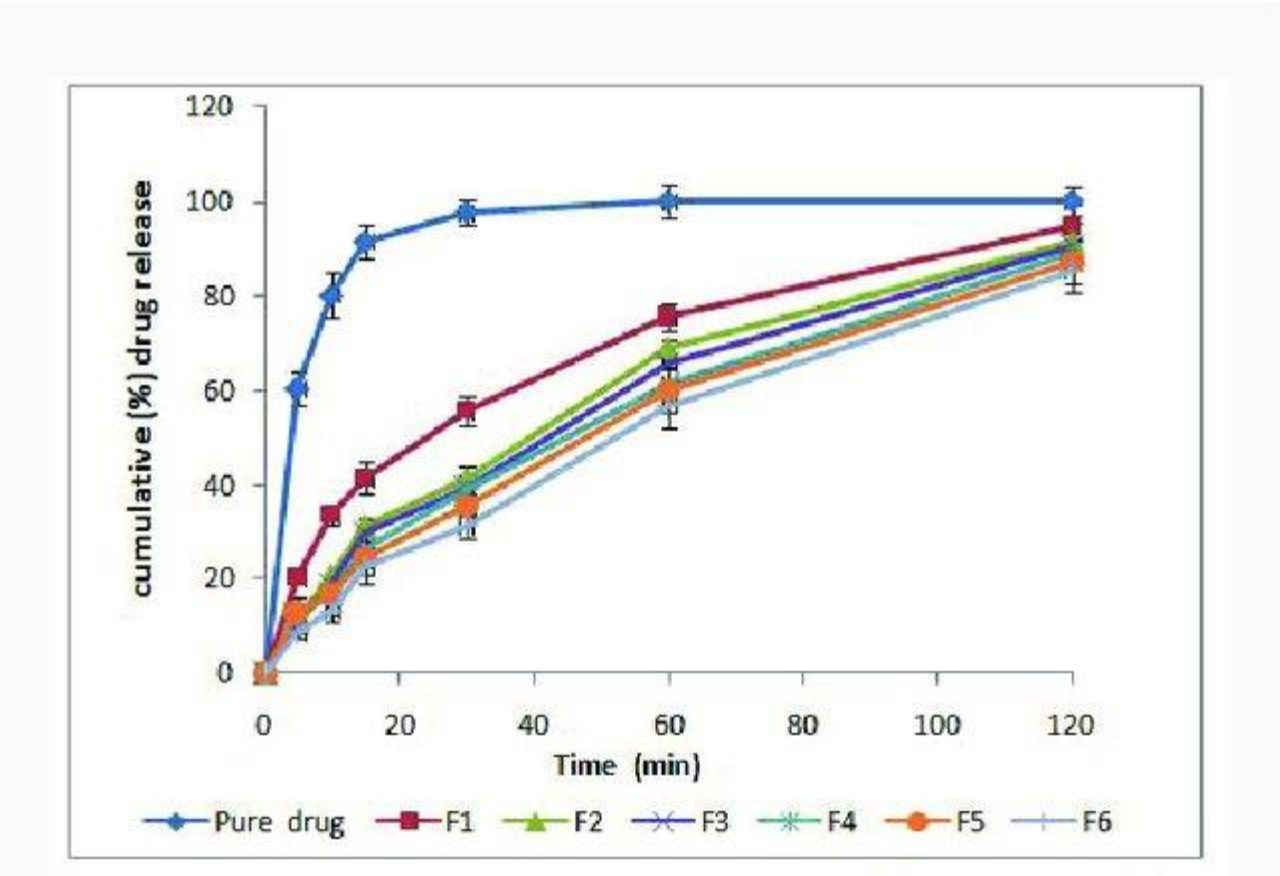


Figure 5: In Vitro Drug Release Profile.

In Vitro Biological Evaluation

Cytotoxicity Assay (MTT)

The anticancer efficacy of PTX-PLGA-NPs was evaluated against MCF-7 and MDA-MB-231 breast cancer cells and compared with free Taxol® and blank PLGA-NPs. Blank NPs showed negligible cytotoxicity (>90% cell viability at all tested concentrations), confirming the polymer's biocompatibility. As shown in Figure 6, both PTX formulations induced dose-dependent cytotoxicity. Crucially, the PTX-PLGA-NPs demonstrated significantly enhanced cytotoxicity compared to free Taxol® after 72 hours of incubation. The calculated IC50 values (Table 2) for PTX-PLGA-NPs were approximately 2.5-fold lower than those for Taxol® in both cell lines (e.g., 4.2 nM vs. 10.5 nM in MDA-MB-231). This indicates a marked increase in cytotoxic potency, likely due to more efficient cellular internalization of the nanoparticle payload compared to passive diffusion of free drug, potentially bypassing efflux pumps.

Table 2: IC50 Values of PTX Formulations After 72h Incubation.

Cell Line	Free Taxol® (IC50, nM)	PTX-PLGA-NPs (IC50, nM)
MCF-7	8.7 ± 0.9	3.5 ± 0.4*
MDA-MB-231	10.5 ± 1.2	4.2 ± 0.5*

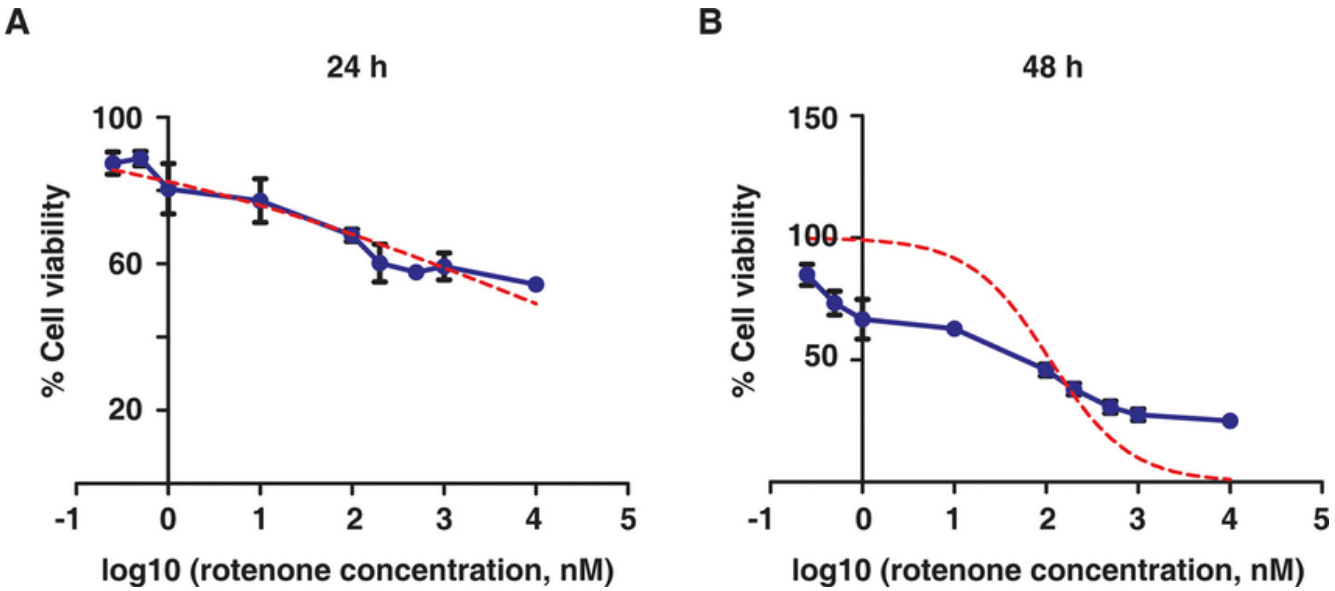
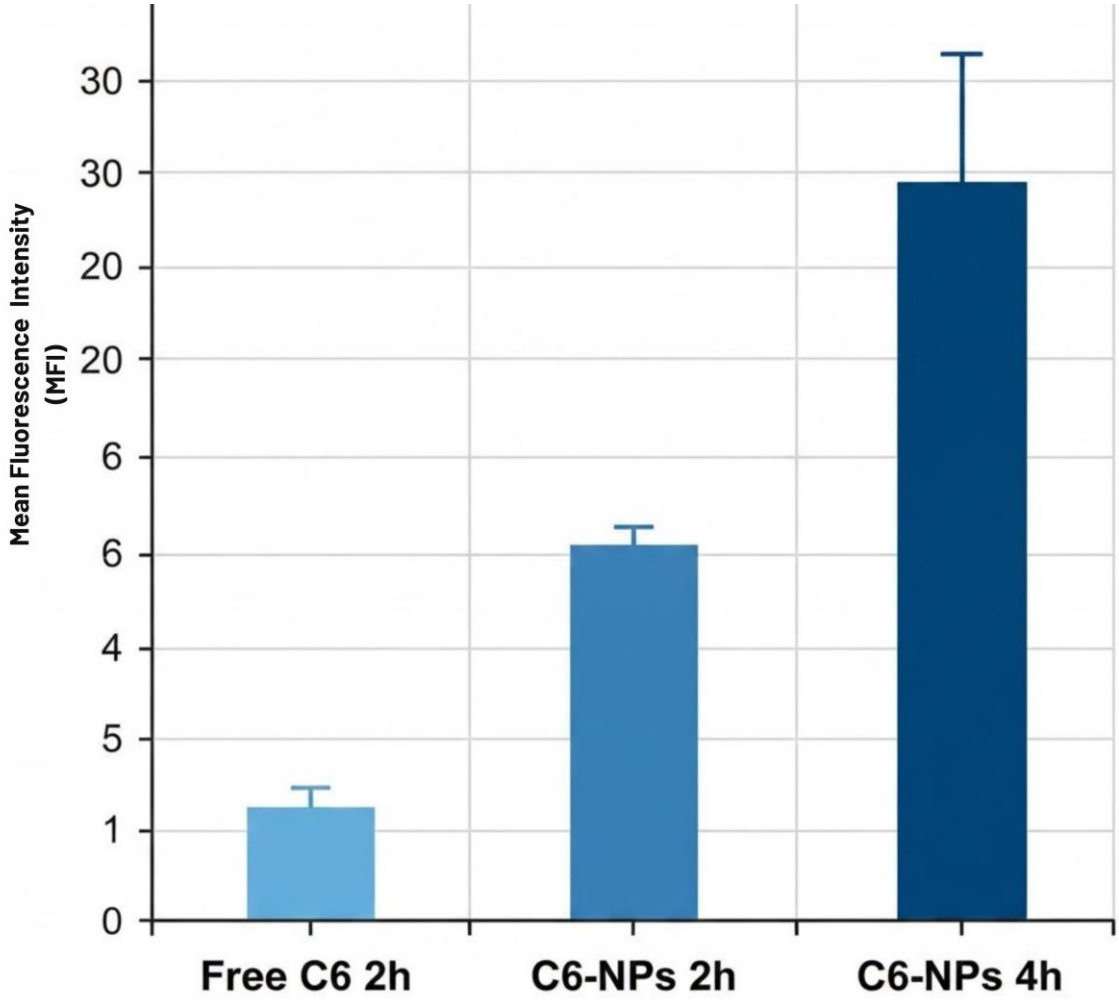


Figure 6: Dose-Response Cytotoxicity Curves.

Cellular Uptake Studies

To elucidate the mechanism behind the enhanced cytotoxicity, cellular uptake was quantified using Coumarin-6-loaded NPs (C6-NPs) as a fluorescent proxy. Flow cytometry analysis (Figure 7A) revealed a time- and concentration-dependent increase in mean fluorescence intensity (MFI) for cells treated with C6-NPs, significantly higher than that for cells treated with an equivalent concentration of free C6 dye after 2 and 4 hours. This confirms the NPs are internalized more efficiently than the free molecule. Fluorescence microscopy (Figure 7B) corroborated these findings. After 2 hours, cells treated with free C6 showed weak, diffuse cytoplasmic fluorescence. In stark contrast, cells treated with C6-NPs displayed intense punctate fluorescence localized in the perinuclear region, indicative of internalization via endocytic pathways and subsequent entrapment in endo-lysosomal vesicles. This enhanced uptake pathway directly facilitates greater intracellular drug accumulation, explaining the lower IC50 values.



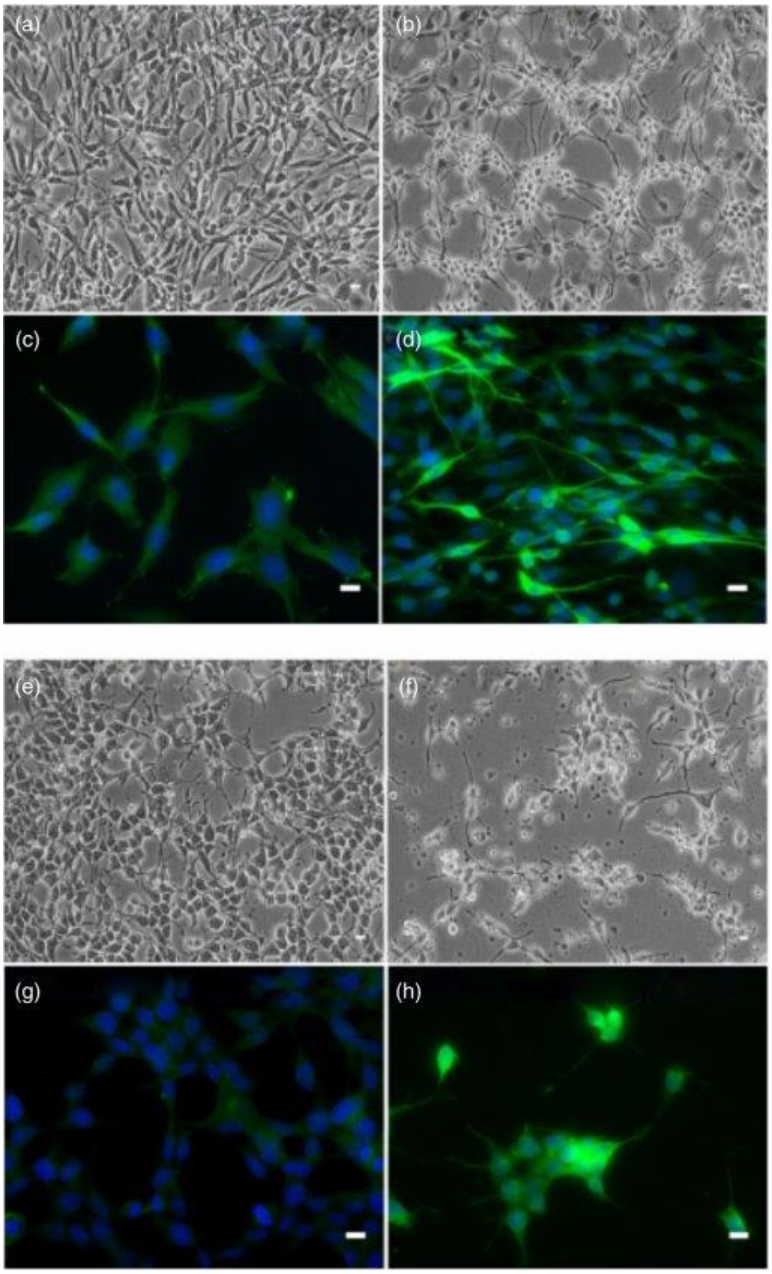


Figure 7: Cellular Uptake Analysis. (A) Bar Graph from Flow Cytometry: Mean Fluorescence Intensity (MFI) on Y-axis, Time/Formulation on X-axis. Bars for C6-NPs at 2h and 4h are substantially taller than for Free C6. (B) Fluorescence Microscopy Images (4 panels): 1. Brightfield image of cells. 2. Cells + Free C6 (dim, diffuse green). 3. Cells + C6-NPs (bright, dotted green spots inside cells). 4. Merged image of panel 3.

DISCUSSION

The results successfully demonstrate the rational design and development of an optimized PTX-loaded PLGA nanoparticle system. The application of DoE was instrumental in efficiently navigating the multivariate formulation space, leading to NPs with ideal characteristics: a small, monodisperse size (~170 nm), high negative zeta potential (-29 mV), and excellent drug loading (EE% >84%). Comprehensive characterization confirmed the amorphization of PTX within the polymer matrix, a key factor for its subsequent release. The *in vitro* drug release profile demonstrated a desirable sustained pattern over one week, which could translate to prolonged therapeutic action *in vivo*. Most significantly, the biological evaluations confirmed the formulation's success. The markedly enhanced cytotoxicity (2.5-fold lower IC50) and the visual/quantitative evidence of superior cellular uptake provided a clear structure-activity relationship. The nanoparticle formulation not only solubilizes PTX but actively promotes its delivery into cancer cells, overcoming a key limitation of the free drug. These promising *in vitro* results provide a strong rationale for further *in vivo* pharmacokinetic and efficacy studies in animal models of breast cancer.

CONCLUSION

This study successfully formulated, optimized, and evaluated a PLGA-based nanoparticle system for the

enhanced delivery of paclitaxel. The application of Quality by Design (QbD) principles through DoE enabled the rational development of nanoparticles with optimal physicochemical properties, including an ideal size for potential passive tumor targeting via the EPR effect, excellent colloidal stability, and high drug payload. The formulation effectively transformed crystalline PTX into an amorphous state within the polymer matrix, facilitating a sustained release pattern. The most compelling evidence of the system's success was demonstrated *in vitro*, where the PTX-PLGA-NPs showed a superior cytotoxic profile and significantly greater cellular internalization compared to the conventional free drug formulation. This enhanced performance is attributed to the nanoparticle's ability to solubilize PTX in an aqueous medium and promote its efficient delivery into cancer cells via endocytic pathways, potentially circumventing efflux mechanisms. By eliminating the need for toxic solubilizing agents like Cremophor EL and providing a platform for controlled release and improved cellular uptake, this PTX-PLGA-NP formulation represents a significant stride toward overcoming the major pharmaceutical limitations of paclitaxel. The promising *in vitro* results lay a strong foundation for subsequent preclinical *in vivo* studies to validate pharmacokinetic advantages, antitumor efficacy, and safety profiles in animal models of breast cancer, ultimately aiming to translate this nanomedicine approach into a more effective and tolerable clinical therapy.

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