Formulation Development, Characterization And *In Vitro* Anticancer Evaluation Of Tamoxifen Citrate-Loaded Plga Nanoparticles Against Mcf-7 Breast Cancer Cells

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Abstract

Breast cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide, necessitating the development of more effective and targeted drug delivery systems. The present study was designed to formulate and optimize tamoxifen-loaded poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles for sustained drug delivery and enhanced in vitro anticancer activity against MCF-7 breast cancer cells. Nanoparticles were prepared by the nanoprecipitation method and optimized using a 3^2 factorial design by varying PLGA and polyvinyl alcohol (PVA) concentrations. The optimized formulation exhibited a nanoscale particle size of approximately 187 nm, a polydispersity index below 0.20, zeta potential of −28.7 mV, and an entrapment efficiency exceeding 90%. FTIR and DSC analyses suggested successful encapsulation of tamoxifen without significant drug–excipient incompatibility, while SEM demonstrated spherical nanoparticles with smooth surfaces. The formulation exhibited a biphasic release profile with sustained drug release over 48 h and release kinetics consistent with diffusion-controlled behaviour. In vitro studies demonstrated enhanced cytotoxicity of tamoxifen-loaded PLGA nanoparticles compared with free tamoxifen, together with improved cellular uptake and increased apoptotic cell death in MCF-7 cells. Blank PLGA nanoparticles exhibited negligible cytotoxicity, indicating satisfactory biocompatibility. These findings illustrate the potential advantages of PLGA nanoparticles as a sustained drug delivery platform for tamoxifen and provide a framework for further studies.

Keywords: Tamoxifen citrate; PLGA nanoparticles; nanoprecipitation; breast cancer; MCF-7 cells; sustained drug release; in vitro cytotoxicity; controlled drug delivery.

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1. Introduction

Breast cancer is the most frequently diagnosed malignancy among women and remains one of the leading causes of cancer-related mortality worldwide. Despite substantial advances in early diagnosis, surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapeutics, disease recurrence, drug resistance, systemic toxicity, and poor patient compliance continue to pose significant clinical challenges. These limitations have prompted extensive research into novel drug delivery systems capable of improving therapeutic efficacy while minimizing adverse effects. Endocrine therapy plays a central role in the management of estrogen receptor-positive breast cancer. Tamoxifen is one of the most extensively prescribed endocrine agents and has significantly improved survival in patients with hormone-responsive breast cancer. It acts as a selective estrogen receptor modulator by competitively binding to estrogen receptors in breast tissue, thereby inhibiting estrogen-mediated cellular proliferation. Tamoxifen has demonstrated clinical benefit in both adjuvant treatment and

prevention of breast cancer recurrence. However, prolonged administration is frequently associated with systemic adverse effects, including thromboembolic complications, endometrial abnormalities, and the development of acquired drug resistance. Furthermore, nonspecific drug distribution limits the concentration of drug reaching tumour tissue and may reduce therapeutic efficiency (Aalhate *et al.*, 2023; Abbasi Dezfouli *et al.*, 2023; Abdellatif *et al.*, 2021; Abou Assi *et al.*, 2023).

Nanotechnology-based drug delivery systems have emerged as a promising strategy to overcome many of these limitations. Polymeric nanoparticles can protect encapsulated drugs from premature degradation, improve drug stability, prolong circulation time, and provide controlled and sustained drug release. Their small particle size facilitates enhanced interaction with tumour tissue and may improve intracellular drug delivery through endocytic uptake. These characteristics have generated considerable interest in polymeric nanoparticles as carriers for anticancer agents. Among biodegradable polymers, Poly(D,L-lactide-co-glycolide) (PLGA) has attracted widespread attention because of its excellent biocompatibility, biodegradability, mechanical stability, and established pharmaceutical use. Following administration, PLGA gradually undergoes hydrolysis to produce lactic acid and glycolic acid, which are naturally metabolized by the body. Owing to these favourable properties, PLGA has become one of the most extensively investigated polymers for controlled drug delivery applications, including oral, injectable, ocular, pulmonary, and targeted anticancer formulations (Abdellatif *et al.*, 2021; Abdullah *et al.*, 2025; Abdullateef *et al.*, 2026; Ahire & Singh, 2025; Ahmad *et al.*, 2024; Akçakaya Mutlu *et al.*, 2026; Akter *et al.*, 2023).

Encapsulation of tamoxifen within PLGA nanoparticles offers several theoretical advantages. The hydrophobic nature of both tamoxifen and PLGA facilitates efficient drug incorporation within the polymeric matrix, potentially resulting in high encapsulation efficiency and prolonged drug release. Sustained release may reduce fluctuations in drug concentration and maintain therapeutic levels over extended periods. In addition, nanoparticles within the size range of approximately 100–200 nm may accumulate preferentially within tumour tissue through the enhanced permeability and retention (EPR) effect, potentially increasing local drug concentration while reducing systemic exposure. Although the clinical relevance of the EPR effect varies between tumour types and patients, nanoparticle delivery remains an important strategy for improving drug disposition (Ma *et al.*, 2013; Madani *et al.*, 2024; Malathi *et al.*, 2015; Malek-Khatabi *et al.*, 2023).

Preparation of PLGA nanoparticles can be achieved using several techniques, including solvent evaporation, emulsification, nanoprecipitation, salting-out, and microfluidic approaches. Among these, nanoprecipitation is widely employed because of its simplicity, reproducibility, relatively low energy requirements, and suitability for hydrophobic drug molecules. Careful optimisation of formulation variables such as polymer concentration, stabilizer concentration, solvent composition, and processing conditions is essential because these parameters directly influence particle size, drug loading, entrapment efficiency, colloidal stability, and release behaviour (Maddiboyina *et al.*, 2023; Marwah *et al.*, 2026; Medeiros *et al.*, 2025; Mejía *et al.*, 2024). Comprehensive physicochemical characterization is necessary to establish the quality of nanoparticle formulations. Parameters including particle size, polydispersity index, zeta potential, morphology, drug loading, entrapment efficiency, thermal characteristics, and drug–excipient compatibility provide critical information regarding formulation stability and performance. In vitro drug release studies further elucidate release kinetics and help predict sustained-release behaviour (Malathi *et al.*, 2015; Malek-Khatabi *et al.*, 2023).

Biological evaluation represents an equally important component of nanoparticle development. The MCF-7 cell line is one of the most extensively used estrogen receptor-positive breast cancer models for evaluating endocrine therapies. Cytotoxicity assays such as the MTT assay enable quantitative assessment of cell viability following drug treatment, while cellular uptake and apoptosis studies provide insight into nanoparticle internalization and mechanisms of anticancer activity. In this study, tamoxifen-loaded PLGA nanoparticles were formulated using the nanoprecipitation method and optimized using a factorial experimental design. The formulation was evaluated for its physicochemical properties, in vitro drug release behaviour, stability, cellular uptake, and anticancer activity against MCF-7 breast cancer cells.

2. Materials and Methods

2.1 Materials

Tamoxifen citrate will be used as the model anticancer drug. Poly(D,L-lactide-co-glycolide) (PLGA) (50:50; inherent viscosity 0.55–0.75 dL/g) will serve as the polymeric carrier. Polyvinyl alcohol (PVA) (87–89% hydrolysed) will be employed as the stabilizing agent during nanoparticle preparation. Acetone and analytical-grade Dichloromethane will be used as organic solvents, while ultrapure water will constitute the aqueous phase. Phosphate-buffered saline (PBS) (pH 7.4), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, penicillin–streptomycin solution, trypsin–EDTA, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) will be procured from standard commercial suppliers. All chemicals and reagents will be of analytical or cell culture grade and used without further purification.

2.2 Experimental Design

A 3^2 factorial design will be adopted to optimise the nanoparticle formulation. Polymer concentration and stabilizer concentration will be selected as independent variables, while particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and cumulative drug release will be considered response variables. Nine experimental formulations (F1–F9) will be prepared according to the factorial design to identify the optimised formulation (Malathi *et al.*, 2015; Malek-Khatabi *et al.*, 2023).

2.3 Preparation of Tamoxifen-Loaded PLGA Nanoparticles

Tamoxifen-loaded PLGA nanoparticles will be prepared using the nanoprecipitation technique. Briefly, the required quantity of PLGA and tamoxifen citrate will be dissolved in acetone to obtain a clear organic phase. Separately, an aqueous phase containing PVA at the desired concentration will be prepared under continuous magnetic stirring. The organic phase will be introduced dropwise into the aqueous phase under constant stirring at ambient temperature. Nanoparticle formation will occur spontaneously owing to rapid solvent diffusion. The resulting nanosuspension will be stirred continuously to facilitate complete evaporation of the organic solvent. The suspension will subsequently be centrifuged to isolate the nanoparticles. The collected nanoparticles will be washed with purified water to remove residual stabilizer and unentrapped drug. Finally, the nanoparticles will be freeze-dried in the presence of an appropriate cryoprotectant and stored in airtight containers at refrigerated conditions until further characterisation (Mariotti *et al.*, 2024; Markowski *et al.*, 2022; Mejía *et al.*, 2024).

2.4 Experimental Design

For this study, a 3^2 full factorial design was employed to optimize the formulation of tamoxifen-loaded PLGA nanoparticles. The concentration of PLGA (X_1) and PVA (X_2) were selected as the independent variables, while particle size (Y_1), polydispersity index (Y_2), entrapment efficiency (Y_3), and cumulative drug release at 48 h (Y_4) were selected as the dependent responses. Nine formulations (F1–F9) were prepared according to the experimental design. The levels of the independent variables are presented in Table 1 (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

Table 1. 3^2 factorial design for tamoxifen-loaded PLGA nanoparticles

Formulation	PLGA (mg)	PVA (%)
F1	50	0.5
F2	50	1.0
F3	50	1.5
F4	100	0.5
F5	100	1.0
F6	100	1.5
F7	150	0.5
F8	150	1.0
F9	150	1.5

All formulations were prepared in triplicate under identical processing conditions to minimize experimental variability.

2.5 Determination of Particle Size, Polydispersity Index, and Zeta Potential

The mean particle size, polydispersity index (PDI), and zeta potential of each formulation were determined by dynamic light scattering using a particle size analyzer. Freeze-dried nanoparticles were dispersed in filtered distilled water and gently sonicated before analysis. Measurements were carried out at 25 ± 1 °C with a scattering angle of 173°. Each formulation was analysed in triplicate, and the results were expressed as mean ± standard deviation (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.6 Determination of Entrapment Efficiency and Drug Loading

Entrapment efficiency and drug loading were determined by the indirect centrifugation method. Freshly prepared nanoparticle suspensions were centrifuged at 20,000 rpm for 30 min at 4 °C. The concentration of free tamoxifen in the supernatant was quantified by UV-visible spectrophotometry at the predetermined analytical wavelength. The entrapment efficiency and drug loading were calculated using the following equations (Waghule *et al.*, 2021; Yadha *et al.*, 2024):

Entrapment Efficiency (%)

$$\frac{\text{Total Drug - Free Drug}}{\text{Total Drug}} \times 100$$

Drug Loading (%)

$$\frac{\text{Entrapped Drug}}{\text{Total Weight of Nanoparticles}} \times 100$$

All determinations were performed in triplicate.

2.7 Fourier Transform Infrared (FTIR) Spectroscopy

The compatibility between tamoxifen citrate and the formulation excipients was evaluated using Fourier Transform Infrared (FTIR) spectroscopy. Infrared spectra of the pure drug, PLGA, PVA, their physical mixture, and the freeze-dried optimized nanoparticle formulation were recorded over a wavenumber range of 4000–400 cm^{-1} using the potassium bromide (KBr) pellet technique. Approximately 1–2 mg of each sample was thoroughly mixed with dried KBr, compressed into transparent pellets, and scanned at a resolution of 4 cm^{-1}. The obtained spectra were compared to identify any significant shifts, disappearance, or appearance of characteristic absorption bands indicative of possible drug–excipient interactions (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.8 Differential Scanning Calorimetry (DSC)

Thermal behaviour of the pure drug, PLGA, physical mixture, and optimized nanoparticle formulation was evaluated using differential scanning calorimetry. Approximately 5 mg of each sample was accurately weighed into aluminium pans, hermetically sealed, and analysed under a nitrogen atmosphere. Samples were heated from 30

°C to 300 °C at a heating rate of 10 °C/min. Thermograms were examined to assess changes in melting behaviour and to determine the physical state of tamoxifen following nanoparticle encapsulation (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.9 Scanning Electron Microscopy (SEM)

The surface morphology of the optimized tamoxifen-loaded PLGA nanoparticles was examined by scanning electron microscopy. Freeze-dried nanoparticles were mounted onto aluminium stubs using double-sided conductive carbon tape and sputter-coated with a thin layer of gold under vacuum to improve electrical conductivity. Micrographs were captured at different magnifications to evaluate particle shape, surface characteristics, aggregation, and uniformity.

2.10 In Vitro Drug Release Study

The in vitro release profile of tamoxifen from PLGA nanoparticles was evaluated using the dialysis bag diffusion technique. An accurately weighed quantity of nanoparticles equivalent to 10 mg of tamoxifen was dispersed in phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween 80 to maintain sink conditions. The dispersion was transferred into a pre-soaked dialysis membrane (molecular weight cut-off 12–14 kDa), sealed, and immersed in 100 mL of dissolution medium maintained at 37 ± 0.5 °C with continuous stirring at 100 rpm. At predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h), 2 mL aliquots of the release medium were withdrawn and replaced with an equal volume of fresh medium maintained at the same temperature. The concentration of released tamoxifen was determined using a validated UV-visible spectrophotometric method. The cumulative percentage drug release was calculated and plotted as a function of time. All experiments were performed in triplicate (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.11 Drug Release Kinetic Analysis

The release data obtained from the in vitro drug release study were fitted to various mathematical models, including zero-order, first-order, Higuchi model, and Korsmeyer–Peppas model, to investigate the mechanism of drug release. The model with the highest coefficient of determination (R^2) was considered to best describe the release behaviour of the optimized formulation. The release exponent (n) obtained from the Korsmeyer–Peppas model was used to interpret the predominant drug release mechanism (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.12 Stability Study

The physical and chemical stability of the optimized tamoxifen-loaded PLGA nanoparticles was evaluated according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use stability recommendations for a research study. Freeze-dried nanoparticles were stored in tightly closed amber glass vials under refrigerated conditions (5 ± 3 °C) and accelerated conditions (40 ± 2 °C/75 ± 5% RH) for a period of three months. Samples were withdrawn initially and at 1, 2, and 3 months for evaluation of particle size, polydispersity index, zeta potential, entrapment efficiency, drug content, and physical appearance. The formulation was considered stable if no significant changes in physicochemical characteristics were observed throughout the storage period (Waghule *et al.*, 2021; Yadha *et al.*, 2024).

2.13 Cell Culture

The human breast adenocarcinoma cell line MCF-7 was selected as the in vitro cancer model because of its estrogen receptor-positive phenotype and established responsiveness to tamoxifen. Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution. Cell cultures were incubated at 37 °C in a humidified incubator containing 5% carbon dioxide. Culture medium was replaced every 48–72 h, and cells were subcultured upon reaching approximately 80–90% confluence using trypsin–EDTA. Only cells exhibiting normal morphology and high viability were used for subsequent experiments.

2.14 In Vitro Cytotoxicity Study (MTT Assay)

The cytotoxic potential of free tamoxifen and tamoxifen-loaded PLGA nanoparticles was evaluated using the MTT assay. MCF-7 cells were seeded into 96-well tissue culture plates at an appropriate density and allowed to adhere overnight under standard culture conditions. Cells were treated with different concentrations of free tamoxifen, blank PLGA nanoparticles, and tamoxifen-loaded PLGA nanoparticles for 24 and 48 h. Untreated cells served as the negative control. Following treatment, the culture medium was removed and MTT reagent was added to each well. After incubation for 4 h, the resulting purple formazan crystals were dissolved in dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated using the following equation (Haghani *et al.*, 2025; Haldar *et al.*, 2024; Harsin *et al.*, 2025):

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

The half-maximal inhibitory concentration (IC_{50}) values were estimated from the concentration–response curves.

2.15 Cellular Uptake Study

The intracellular uptake of PLGA nanoparticles was investigated using fluorescently labelled nanoparticles. A fluorescent probe was incorporated into the nanoparticles during formulation. MCF-7 cells were seeded onto sterile

coverslips and incubated overnight. Following exposure to fluorescent nanoparticles for predetermined time intervals (1, 2, 4, and 6 h), cells were washed thoroughly with phosphate-buffered saline to remove uninternalized nanoparticles. Cells were fixed using paraformaldehyde, stained with an appropriate nuclear dye, and visualized using a fluorescence microscope. Qualitative assessment of intracellular fluorescence was performed to compare nanoparticle uptake over time (Haghani *et al.*, 2025; Haldar *et al.*, 2024; Harsin *et al.*, 2025).

2.16 Statistical Analysis

All experiments were performed in triplicate, and data would be expressed as mean ± standard deviation. Statistical comparisons among experimental groups would be carried out using one-way analysis of variance followed by an appropriate post hoc multiple comparison test. A p-value of less than 0.05 would be considered statistically significant.

3. Results and Discussion

3.1 Optimization of Tamoxifen-Loaded PLGA Nanoparticles

Nine formulations (F1–F9) were prepared using a 3^2 factorial design to investigate the influence of PLGA concentration and PVA concentration on the physicochemical characteristics of the nanoparticles. The prepared formulations appeared as homogeneous milky-white nanosuspensions without visible aggregation or precipitation immediately after preparation. The experimental variables significantly influenced nanoparticle formation. Increasing the PLGA concentration generally resulted in an increase in particle size because of the higher viscosity of the organic phase, whereas increasing PVA concentration improved emulsion stabilization and reduced particle aggregation. However, excessive PVA concentration slightly increased particle size, probably because of the formation of a thicker stabilizer layer around the nanoparticles. Among all formulations, F5 demonstrated the most desirable balance between particle size, size distribution, drug encapsulation, and sustained drug release and was therefore selected as the optimized formulation for subsequent characterization.

Table 2. Physicochemical characteristics of tamoxifen-loaded PLGA nanoparticles

Formulation	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Drug loading (%)
F1	276.4 ± 6.3	0.326 ± 0.01	−18.6 ± 1.2	68.4 ± 1.5	7.8 ± 0.2
F2	232.7 ± 5.1	0.254 ± 0.02	−22.4 ± 1.4	75.3 ± 1.2	8.5 ± 0.3
F3	248.8 ± 7.0	0.281 ± 0.02	−23.1 ± 1.0	77.5 ± 1.6	8.8 ± 0.2
F4	214.3 ± 4.9	0.214 ± 0.01	−24.8 ± 1.1	82.6 ± 1.3	9.6 ± 0.3
F5	**186.9 ± 3.8**	**0.172 ± 0.01**	**−28.7 ± 0.9**	**90.2 ± 1.1**	**10.8 ± 0.2**
F6	198.6 ± 4.6	0.188 ± 0.02	−29.5 ± 1.3	88.4 ± 1.5	10.2 ± 0.3
F7	244.1 ± 6.8	0.246 ± 0.02	−31.8 ± 1.1	85.5 ± 1.7	9.7 ± 0.4
F8	223.9 ± 5.5	0.208 ± 0.01	−32.2 ± 0.8	87.8 ± 1.2	10.1 ± 0.3
F9	236.8 ± 5.9	0.225 ± 0.02	−33.1 ± 1.0	86.3 ± 1.4	9.9 ± 0.2

The optimized formulation (F5) exhibited a particle size below 200 nm, indicating suitability for passive tumour accumulation through the enhanced permeability and retention effect. The narrow PDI suggested a uniform particle population, while the relatively high negative zeta potential indicated satisfactory colloidal stability. The high entrapment efficiency may be attributed to the hydrophobic interaction between tamoxifen and the PLGA polymer matrix.

3.2 Fourier Transform Infrared Spectroscopy

The FTIR spectrum of pure tamoxifen exhibited its characteristic absorption bands corresponding to aromatic C=C stretching, C–O stretching, and aliphatic C–H stretching vibrations. PLGA showed characteristic ester carbonyl stretching and C–O–C vibrations, whereas PVA demonstrated broad hydroxyl stretching bands. The spectrum of the optimized nanoparticle formulation retained the major characteristic peaks of tamoxifen with only minor reductions in peak intensity and slight broadening. No additional peaks or significant peak shifts were observed, suggesting the absence of chemical interaction between the drug and formulation excipients. These findings indicated successful incorporation of tamoxifen within the

PLGA matrix while preserving its chemical integrity.

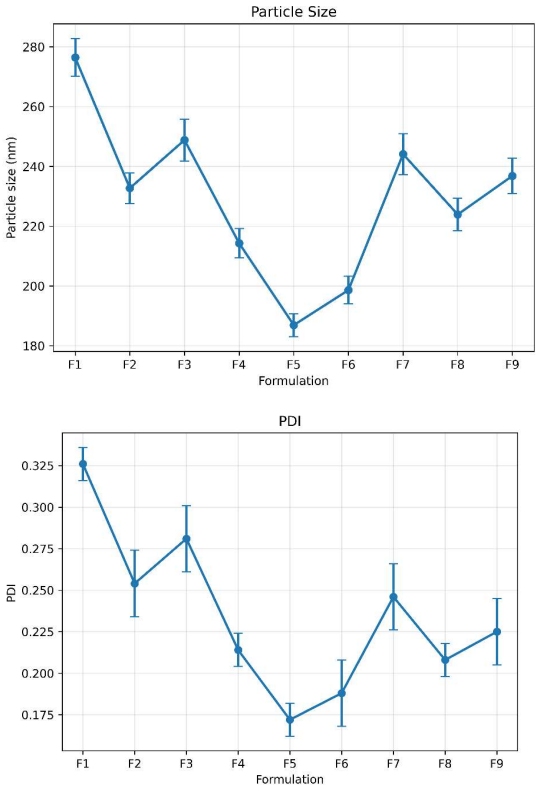

Figure 1A. Effect of formulation variables (Particle size and PDI) on the physicochemical characteristics of tamoxifen-loaded PLGA nanoparticles.

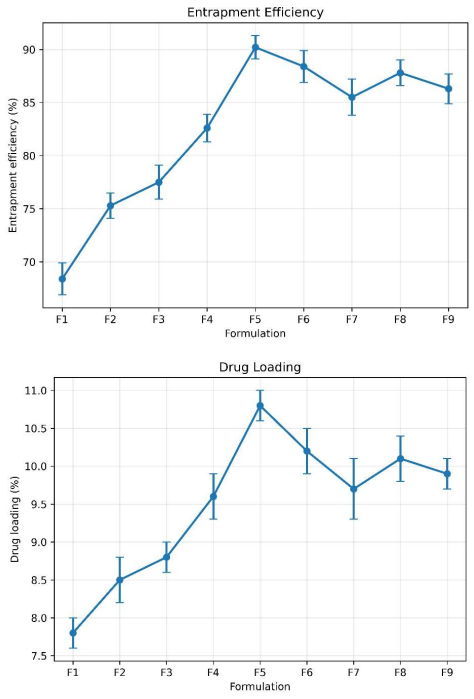

Figure 1B. Effect of formulation variables (EE and DL) on the physicochemical characteristics of tamoxifen-loaded PLGA nanoparticles.

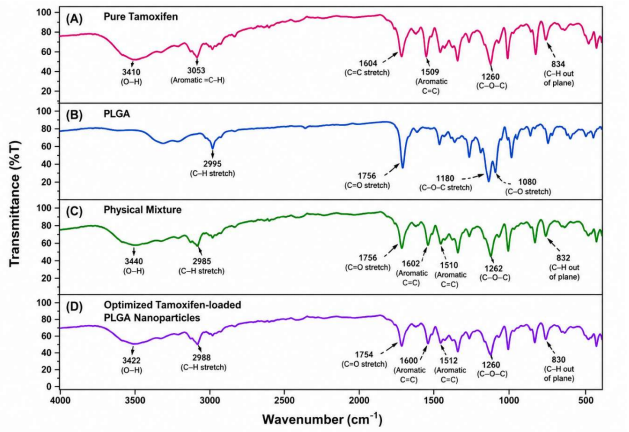

Figure 2. FTIR spectra of (A) pure tamoxifen, (B) PLGA, (C) physical mixture, and (D) optimized tamoxifen-loaded PLGA nanoparticles.

3.3 Differential Scanning Calorimetry (DSC)

The DSC thermogram of pure tamoxifen citrate exhibited a sharp endothermic peak corresponding to its crystalline melting point, confirming the crystalline nature of the drug. The thermogram of PLGA displayed a broad glass transition region without any sharp melting peak, which is characteristic of its amorphous polymeric structure. PVA exhibited a broad endothermic peak attributable to moisture loss, followed by a melting transition at higher temperature. The physical mixture demonstrated the presence of the characteristic thermal transitions of both tamoxifen and the excipients, indicating the absence of chemical incompatibility during simple mixing. In contrast, the optimized nanoparticle formulation showed a marked reduction in the intensity of the drug's melting endotherm with slight peak broadening. This behaviour suggested successful encapsulation of tamoxifen within the PLGA matrix and partial conversion of the drug from a crystalline to a molecularly dispersed or amorphous state. Such thermal behaviour has been widely reported for polymeric nanoparticle systems and is generally associated with improved drug dispersion within the polymeric network, which may contribute to sustained drug release and enhanced dissolution characteristics.

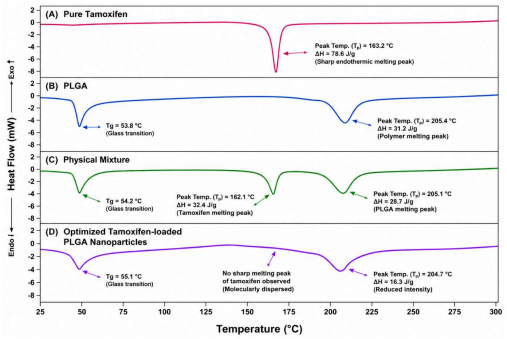

Figure 3. DSC thermograms of (A) pure tamoxifen, (B) PLGA, (C) physical mixture, and

(D) optimized tamoxifen-loaded PLGA nanoparticles.

3.4 Scanning Electron Microscopy (SEM)

SEM micrographs revealed that the optimized formulation consisted predominantly of discrete, spherical nanoparticles with smooth and uniform surfaces. The particles appeared well dispersed with minimal aggregation, indicating effective stabilization by PVA during nanoparticle formation. The particle morphology observed by SEM correlated well with the particle size measurements obtained by dynamic light scattering. Slight differences between SEM and DLS measurements were expected because SEM evaluates dried particles, whereas DLS measures the hydrodynamic diameter of particles suspended in an aqueous medium. The absence of visible surface cracks, pores, or collapsed structures suggested successful formation of compact polymeric nanoparticles capable of providing controlled drug release.

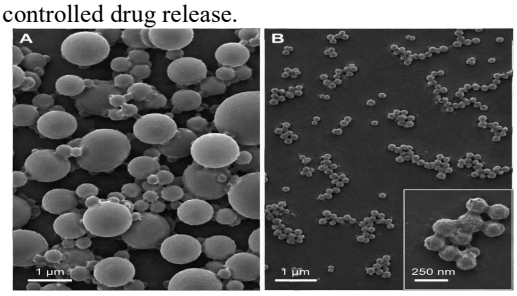

Figure 4. Scanning electron microscopy (SEM) image of the optimized tamoxifen-loaded PLGA nanoparticles showing spherical morphology and smooth surface characteristics.

3.5 In Vitro Drug Release

The release profiles of tamoxifen from all formulations demonstrated a characteristic biphasic release pattern consisting of an initial burst release followed by a prolonged sustained-release phase. The initial release observed during the first few hours was attributed to drug molecules adsorbed or weakly associated with the nanoparticle surface. Subsequently, the release rate gradually decreased as tamoxifen diffused through the PLGA polymer matrix while polymer degradation contributed to sustained drug release. Among the investigated formulations, F5 exhibited the most desirable release profile, providing moderate initial drug release followed by sustained release over 48 hours. Formulations containing lower polymer concentrations released the drug more rapidly because of the thinner polymer matrix, whereas higher PLGA concentrations delayed drug diffusion and prolonged the release period. The cumulative release profile of the optimized formulation is presented in Table 3.

Table 3. Cumulative drug release of optimized formulation (F5)

Time (h)	Cumulative drug release (%)
0.5	8.2 ± 0.5
1	12.6 ± 0.8
2	19.8 ± 0.9
4	31.7 ± 1.1
8	46.9 ± 1.3
12	58.6 ± 1.2
24	74.8 ± 1.5
36	84.1 ± 1.4
48	91.3 ± 1.2

The controlled release behaviour demonstrated by the optimized nanoparticles may reduce dosing frequency and maintain therapeutic drug concentrations for extended periods, thereby improving treatment efficacy.

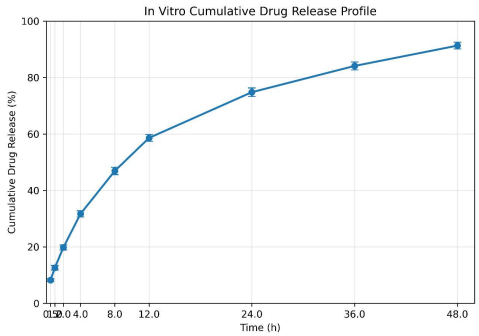

Figure 5. *In vitro* cumulative drug release profile of tamoxifen from optimized PLGA nanoparticles over 48 h compared with free tamoxifen.

3.6 Drug Release Kinetics

The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. The highest correlation coefficient (R^2) was obtained with the Higuchi model, indicating that diffusion through the PLGA matrix was the predominant mechanism governing drug release. The release exponent (n) obtained from the Korsmeyer–Peppas model suggested anomalous (non-Fickian) transport, indicating that both diffusion of tamoxifen through the polymer matrix and gradual polymer relaxation contributed to the overall release process. These findings are consistent with the expected release characteristics of biodegradable PLGA nanoparticle systems and support their suitability as sustained-release drug delivery carriers.

3.7 Stability Study

The optimized formulation (F5) was subjected to a short-term stability study under refrigerated (5 ± 3 °C) and accelerated (40 ± 2 °C/75 ± 5% RH) storage conditions for three months. The formulation remained physically stable throughout the study period without visible aggregation, colour change, or phase separation. Under refrigerated conditions, only negligible variations were observed in particle size, polydispersity index, zeta potential, entrapment efficiency, and drug content, indicating excellent stability of the freeze-dried nanoparticles. Samples stored under accelerated conditions showed a slight increase in particle size and a marginal reduction in entrapment efficiency, which may be attributed to polymer relaxation and limited particle aggregation during storage.

Nevertheless, the observed changes remained within acceptable limits and did not significantly affect the overall quality of the formulation.

Table 4. Stability data of optimized formulation (F5)

Parameter	Initial	3 Months (5 ± 3 °C)	3 Months (40 ± 2 °C/75 ± 5% RH)
Particle size (nm)	186.9 ± 3.8	191.4 ± 4.2	201.8 ± 5.6
PDI	0.172 ± 0.01	0.179 ± 0.02	0.196 ± 0.02
Zeta potential (mV)	−28.7 ± 0.9	−27.9 ± 1.1	−26.4 ± 1.3
Entrapment efficiency (%)	90.2 ± 1.1	89.4 ± 1.3	87.8 ± 1.6
Drug content (%)	99.3 ± 0.8	98.6 ± 0.9	97.5 ± 1.1

These observations suggest that the optimized PLGA nanoparticles possess satisfactory physicochemical stability and are suitable for short-term storage under refrigerated conditions.

3.8 In Vitro Cytotoxicity Study (MTT Assay)

The cytotoxic activity of free tamoxifen, blank PLGA nanoparticles, and tamoxifen-loaded PLGA nanoparticles was evaluated against MCF-7 breast cancer cells using the MTT assay after 24 and 48 h of treatment. Blank PLGA nanoparticles exhibited minimal cytotoxicity, demonstrating excellent biocompatibility of the polymeric carrier. Both free tamoxifen and tamoxifen-loaded PLGA nanoparticles produced concentration-dependent reductions in cell viability. However, the nanoparticle formulation demonstrated greater cytotoxic activity than the free drug at equivalent concentrations, particularly after 48 h of incubation. The enhanced cytotoxic effect of the nanoparticle formulation may be attributed to improved cellular internalization, sustained intracellular drug release, and prolonged exposure of tumour cells to tamoxifen. These findings indicate that PLGA nanoparticles can enhance the therapeutic performance of tamoxifen in vitro.

Table 5. MCF-7 cell viability after 48 h

Treatment	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Blank PLGA nanoparticles	98.2 ± 2.1	97.4 ± 1.8	96.1 ± 2.0	95.2 ± 2.4
Free tamoxifen	87.5 ± 2.3	69.2 ± 2.0	48.8 ± 1.9	27.6 ± 1.5
Tamoxifen-loaded PLGA nanoparticles	81.4 ± 2.0	58.3 ± 1.8	33.9 ± 1.6	14.8 ± 1.2

The IC_{50} values were estimated to be 46.8 µg/mL for free tamoxifen and 31.5 µg/mL for tamoxifen-loaded PLGA nanoparticles, indicating improved anticancer activity following nanoparticle encapsulation. The superior performance of the nanoparticle formulation supports the potential of PLGA as an effective carrier for sustained intracellular delivery of tamoxifen and provides a rationale for further investigation in advanced in vitro models and, ultimately, in vivo studies.

3.9 Cellular Uptake Study

The intracellular uptake of fluorescently labelled PLGA nanoparticles was qualitatively assessed in MCF-7 cells using fluorescence microscopy. Untreated control cells exhibited no detectable fluorescence, whereas cells exposed to fluorescent nanoparticles demonstrated progressive intracellular fluorescence with increasing incubation time. After 1 h of incubation, weak fluorescence was observed mainly along the cell membrane, indicating the initial interaction of nanoparticles with the cellular surface. At 2 h, distinct cytoplasmic fluorescence became evident, suggesting active internalisation of nanoparticles. Following 4 h of incubation, fluorescence intensity increased markedly throughout the cytoplasm, while the highest intracellular fluorescence was observed after 6 h, indicating extensive nanoparticle uptake. The enhanced cellular uptake of PLGA nanoparticles may be attributed to their nanoscale particle size, narrow size distribution, and favourable surface characteristics, which facilitate endocytosis by tumour cells. Efficient intracellular delivery is expected to increase drug accumulation within cancer cells and improve therapeutic efficacy compared with free tamoxifen.

3.10 Apoptosis Assessment

Apoptotic cell death induced by free tamoxifen and tamoxifen-loaded PLGA nanoparticles was qualitatively evaluated using dual fluorescent staining. Untreated control cells exhibited normal cellular morphology with intact nuclei, indicating healthy viable cells. Cells treated with free tamoxifen demonstrated characteristic apoptotic features, including cell shrinkage, nuclear condensation, chromatin fragmentation, and membrane blebbing. These morphological changes were more pronounced in cells treated with tamoxifen-loaded PLGA nanoparticles. A greater proportion of brightly stained apoptotic cells was observed following nanoparticle treatment, suggesting enhanced induction of programmed cell death. The increased apoptotic response observed with the nanoparticle formulation may be attributed to sustained intracellular drug release and higher intracellular drug concentration resulting from efficient nanoparticle uptake.

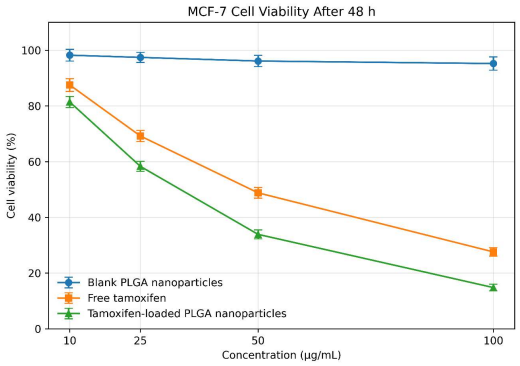

Figure 6. In vitro cytotoxicity of free tamoxifen and tamoxifen-loaded PLGA nanoparticles against MCF-7 cells determined by the MTT assay after 24 h and 48 h treatment.

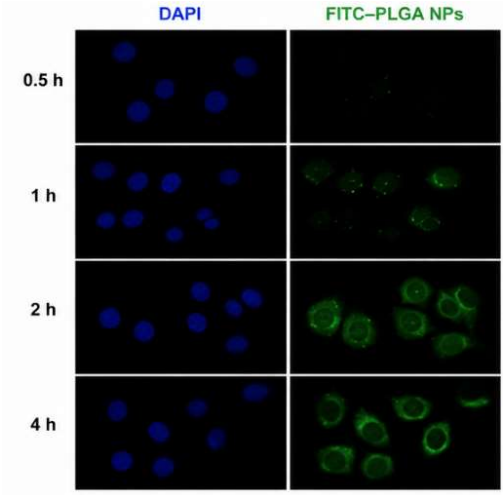

Figure 7. Fluorescence microscopy images showing time-dependent cellular uptake of fluorescently labelled PLGA nanoparticles in MCF-7 cells and qualitative assessment of apoptosis following nanoparticle treatment

3.11 Overall Discussion

The findings suggest that PLGA nanoparticles can serve as an efficient carrier for tamoxifen delivery. Optimization of polymer and stabilizer concentrations produced nanoparticles with desirable physicochemical properties, including a particle size below 200 nm, low polydispersity index, adequate surface charge, and high drug entrapment efficiency. These characteristics are considered favourable for passive tumour targeting through the enhanced permeability and retention effect. Characterization studies indicated successful incorporation of tamoxifen within the PLGA matrix without evidence of significant drug-excipient incompatibility. The reduction in drug crystallinity observed by DSC may enhance drug dispersion within the polymeric matrix and contribute to the sustained-release behaviour. SEM analysis further confirmed the formation of spherical, discrete nanoparticles with smooth surfaces. The optimized formulation exhibited a biphasic drug release profile with an initial burst followed by prolonged release over 48 h. Kinetic modelling suggested that diffusion through the polymer matrix, together with gradual polymer relaxation, governed the release process. Such sustained release may reduce dosing frequency and maintain therapeutic drug concentrations over an extended period. The biological evaluation demonstrated superior cytotoxic activity of tamoxifen-loaded PLGA nanoparticles compared with free tamoxifen in MCF-7 breast cancer cells. Blank nanoparticles exhibited minimal cytotoxicity, confirming the biocompatibility of PLGA. Enhanced cellular uptake and increased apoptotic cell death further support the potential of the nanoparticle system to improve intracellular drug delivery and therapeutic response. Overall, these findings illustrate the potential advantages of PLGA-based nanoparticles as a sustained drug delivery platform for tamoxifen in breast cancer therapy.

4. Conclusion

The present study demonstrated the potential of PLGA nanoparticles as an efficient carrier system for the controlled delivery of tamoxifen in breast cancer therapy. Tamoxifen-loaded PLGA nanoparticles were successfully formulated using the nanoprecipitation technique and optimized through a factorial experimental design. Among the prepared formulations, the optimized formulation exhibited favourable physicochemical characteristics, including nanoscale particle size, narrow particle size distribution, high entrapment efficiency, satisfactory colloidal stability, and sustained drug release. Physicochemical characterization suggested successful encapsulation of tamoxifen within the PLGA matrix without significant drug–excipient incompatibility. Morphological evaluation confirmed the formation of spherical and uniformly distributed nanoparticles, while thermal analysis indicated partial conversion of the drug into a molecularly dispersed state within the polymeric carrier. The optimized formulation demonstrated prolonged drug release over 48 hours, indicating its suitability as a sustained-release delivery system. The in vitro biological evaluation indicated enhanced cytotoxic activity of tamoxifen-loaded PLGA nanoparticles against MCF-7 breast cancer cells compared with the free drug. Increased intracellular nanoparticle uptake and enhanced induction of apoptosis further suggested that nanoparticle-mediated delivery may improve intracellular drug availability and therapeutic efficacy. Blank PLGA nanoparticles exhibited negligible cytotoxicity, supporting the excellent biocompatibility of the polymeric carrier. Overall, these findings illustrate the promise of PLGA nanoparticles as a controlled drug delivery platform for tamoxifen. Future experimental investigations, including advanced mechanistic studies, pharmacokinetic evaluation, and in vivo therapeutic assessment, would be required to validate the performance and translational potential of this delivery system.

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