



Original Research Paper

Development and Evaluation of Folate-conjugated elacestrant-Loaded PLGA Nanoparticles for Targeted therapy of Estrogen receptor positive Breast Cancer

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Abstract

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide, with estrogen receptor-positive (ER+) breast cancer accounting for nearly 70% of all diagnosed cases. Elacestrant, a recently approved oral selective estrogen receptor degrader (SERD), has shown promising therapeutic efficacy in ER-positive breast cancer; however, its clinical performance may be limited by poor aqueous solubility and non-specific systemic distribution. The present study aimed to develop folate-conjugated polymeric nanoparticles of Elacestrant for targeted delivery to ER-positive breast cancer cells. Elacestrant-loaded PLGA nanoparticles were prepared using the emulsion solvent evaporation method and subsequently functionalized with folic acid via EDC/NHS-mediated conjugation. The optimized nanoparticles exhibited a particle size of 168.2 ± 5.3 nm, polydispersity index of 0.205 ± 0.03 , zeta potential of -18.9 ± 1.5 mV, entrapment efficiency of $82.8 \pm 2.4\%$, and folate conjugation efficiency of $71.6 \pm 3.2\%$. The formulation demonstrated sustained drug release over 48 h and followed Korsmeyer-Peppas release kinetics. Cellular studies revealed significantly enhanced uptake of folate-conjugated nanoparticles in MCF-7 cells compared with free drug and non-targeted

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nanoparticles. The targeted formulation exhibited superior cytotoxicity, increased intracellular reactive oxygen species generation, and markedly enhanced apoptosis induction. Furthermore, the nanoparticles demonstrated improved selectivity toward cancer cells while maintaining acceptable stability during storage. Collectively, these findings suggest that folate-conjugated Elacestrant-loaded PLGA nanoparticles may serve as a promising targeted nanotherapeutic strategy for the treatment of estrogen receptor-positive breast cancer.

Keywords: Elacestrant; PLGA nanoparticles; folate conjugation; estrogen receptor-positive breast cancer; targeted drug delivery; selective estrogen receptor degrader.

1. Introduction

Breast cancer remains the most frequently diagnosed malignancy among women and is a major cause of cancer-associated mortality worldwide. According to recent global cancer statistics, breast cancer accounts for a substantial proportion of newly diagnosed cancer cases and continues to pose a significant public health burden despite considerable advances in diagnosis and treatment. Among the various molecular subtypes, estrogen receptor-positive (ER+) breast cancer represents approximately 70% of all breast cancer cases and is primarily dependent on estrogen signalling for tumor growth and progression. Endocrine therapy has long served as the cornerstone of treatment for ER-positive breast cancer. Conventional endocrine agents, including selective estrogen receptor modulators, aromatase inhibitors, and selective estrogen receptor degraders (SERDs), have significantly improved patient outcomes. However, prolonged treatment frequently leads to endocrine resistance, disease recurrence, and metastatic progression. Consequently, there remains a continuous need for improved therapeutic strategies capable of enhancing drug efficacy while minimizing systemic toxicity (Zablon *et al.*, 2026; Zhang *et al.*, 2026; Zhou *et al.*, 2026). Elacestrant is a novel oral selective estrogen receptor degrader that has recently gained considerable attention due to its ability to induce estrogen receptor degradation and inhibit estrogen-dependent signalling

pathways. Unlike earlier SERDs, Elacestrant exhibits favourable oral bioavailability and has demonstrated significant clinical benefits in patients with advanced ER-positive and HER2-negative breast cancer, particularly in endocrine-resistant disease. Nevertheless, the therapeutic performance of Elacestrant may still be limited by its poor aqueous solubility, potential off-target distribution, and inadequate tumor-specific accumulation. Non-selective systemic exposure may contribute to dose-related adverse effects and reduce therapeutic efficiency (Jacobson, 2022; Olivier & Prasad, 2022; Sanchez *et al.*, 2022).

Nanotechnology-based drug delivery systems have emerged as promising approaches for overcoming several limitations associated with conventional anticancer therapies. Polymeric nanoparticles, particularly those composed of poly(D,L-lactide-co-glycolide) (PLGA), have attracted substantial interest owing to their excellent biocompatibility, biodegradability, controlled drug release properties, and regulatory acceptance. Encapsulation of hydrophobic drugs within PLGA nanoparticles can improve drug dispersion, protect the encapsulated drug from premature degradation, and facilitate sustained therapeutic delivery. In addition to passive tumor targeting through the enhanced permeability and retention effect, active targeting strategies can further improve therapeutic selectivity. Folate

receptor-mediated targeting is one of the most extensively investigated approaches in cancer nanomedicine. Folate receptors are overexpressed in several malignant cells, including various breast cancer subtypes, while their expression in most normal tissues remains relatively low. Surface modification of nanoparticles with folic acid can therefore facilitate receptor-mediated endocytosis, resulting in enhanced intracellular drug accumulation and improved anticancer efficacy (Feng *et al.*, 2022; Franca *et al.*, 2022; Paiva *et al.*, 2022; Rajana *et al.*, 2022).

The combination of Elacestrant therapy with folate-targeted nanoparticle delivery may provide multiple therapeutic advantages, including sustained drug release, enhanced cellular uptake, improved selectivity toward tumor cells, and reduced systemic toxicity. Despite the recent clinical importance of Elacestrant, studies investigating its targeted nanoformulation remain extremely limited (Olivier & Prasad, 2022; Sanchez *et al.*, 2022; Singh *et al.*, 2025). Therefore, the present investigation was undertaken to develop folate-conjugated PLGA nanoparticles of Elacestrant and evaluate their physicochemical characteristics, drug release behaviour, cellular uptake, cytotoxic potential, intracellular reactive oxygen species generation, apoptosis-inducing ability, and formulation stability in ER-positive breast cancer cells. The study aimed to establish a targeted nanotherapeutic platform capable of improving the therapeutic performance of Elacestrant for breast cancer management.

2. Materials and Methods

2.1 Materials

Elacestrant was procured from MedChemExpress (USA). Poly(D,L-lactide-co-glycolide) (PLGA 50:50; molecular weight 30,000–60,000 Da), polyvinyl alcohol (PVA; 87–89%

hydrolyzed), folic acid, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), coumarin-6, dichloromethane (DCM), ethyl acetate, dimethyl sulfoxide (DMSO), MTT reagent, dichlorofluorescein diacetate (DCFH-DA), and Annexin V-FITC/propidium iodide apoptosis detection kit were purchased from Sigma-Aldrich (USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, phosphate-buffered saline (PBS), penicillin, and streptomycin were obtained from Gibco (USA). Human breast adenocarcinoma (MCF-7) and normal mammary epithelial (MCF-10A) cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, India. All chemicals and solvents used in the study were of analytical grade.

2.2 Preformulation Studies

2.2.1 Solubility Studies

The solubility of Elacestrant was determined in various solvents and excipients to select suitable components for nanoparticle preparation. Excess drug was added to 5 mL of distilled water, phosphate buffer (pH 6.8 and pH 7.4), methanol, ethanol, dichloromethane, ethyl acetate, and DMSO in sealed glass vials. The mixtures were shaken at $25 \pm 1^\circ\text{C}$ for 72 h using an orbital shaker operating at 100 rpm. Samples were centrifuged at 10,000 rpm for 15 min, filtered through a 0.22 μm membrane filter, appropriately diluted, and analyzed using a UV-visible spectrophotometer at the predetermined wavelength of maximum absorption (Beraldo-Araújo *et al.*, 2022; Haimhoffer *et al.*, 2021; Ostróžka-Ciešlik, 2025; Todryk *et al.*, 2026).

2.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

Drug-excipient compatibility was evaluated by FTIR spectroscopy. Spectra of pure Elacestrant, PLGA, folic acid, physical

mixtures, and optimized nanoparticles were recorded using an FTIR spectrophotometer within the range of 4000–400 cm^{-1} employing the potassium bromide pellet method (Beraldo-Araújo *et al.*, 2022; Haimhoffer *et al.*, 2021; Ostróžka-Cieślik, 2025; Todryk *et al.*, 2026).

2.2.3 Differential Scanning Calorimetry (DSC)

Thermal behavior of the drug and formulations was investigated using differential scanning calorimetry. Approximately 5 mg of each sample was accurately weighed into aluminum pans and heated from 30°C to 300°C at a heating rate of 10°C/min under a nitrogen atmosphere (Beraldo-Araújo *et al.*, 2022; Haimhoffer *et al.*, 2021; Ostróžka-Cieślik, 2025; Todryk *et al.*, 2026).

2.2.4 Powder X-Ray Diffraction (PXRD)

Crystalline characteristics of Elacestrant before and after encapsulation were examined using an X-ray diffractometer equipped with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). Samples were scanned over a diffraction angle (2θ) range of 5°–60° (Beraldo-Araújo *et al.*, 2022; Haimhoffer *et al.*, 2021; Ostróžka-Cieślik, 2025; Todryk *et al.*, 2026).

2.3 Experimental Design and Optimization

A three-factor, three-level Box-Behnken design (BBD) was employed to optimize formulation variables affecting nanoparticle characteristics. Design-Expert® software (Version 13, Stat-Ease Inc., USA) was utilized for experimental design generation and statistical analysis. The independent variables selected were (Beraldo-Araújo *et al.*, 2022; Haimhoffer *et al.*, 2021; Ostróžka-Cieślik, 2025; Todryk *et al.*, 2026):

- A: PLGA concentration (50–100 mg)
- B: PVA concentration (0.5–1.5% w/v)

- C: Sonication time (2–6 min)

The dependent responses included:

- Y₁: Particle size (nm)
- Y₂: Entrapment efficiency (%)
- Y₃: Drug release after 48 h (%)

Quadratic polynomial equations were generated, and response surface methodology was applied to determine the optimized formulation.

2.4 Preparation of Elacestrant-Loaded Polymeric Nanoparticles

Elacestrant-loaded polymeric nanoparticles were prepared by the emulsion solvent evaporation method. Briefly, accurately weighed quantities of Elacestrant and PLGA were dissolved in 5 mL of a dichloromethane:ethyl acetate mixture (1:1 v/v) to obtain the organic phase. This organic phase was slowly added to 20 mL aqueous PVA solution under magnetic stirring at 1000 rpm. The resulting coarse emulsion was subjected to probe sonication at 40% amplitude for the predetermined duration according to the experimental design. The formed oil-in-water nanoemulsion was stirred continuously for 4 h at room temperature to allow complete evaporation of organic solvents. Nanoparticles were recovered by centrifugation at 15,000 rpm for 30 min at 4°C and washed three times with distilled water to remove excess stabilizer and untrapped drug. The final nanoparticle dispersion was freeze-dried in the presence of mannitol (5% w/v) as cryoprotectant and stored in airtight containers until further studies (Aanisah *et al.*, 2023; Barcena *et al.*, 2023; Galarreta-Rodriguez *et al.*, 2023; Garcia *et al.*, 2023).

2.5 Surface Conjugation with Folic Acid

Folate conjugation was carried out using carbodiimide chemistry. Briefly, optimized PLGA nanoparticles were dispersed in MES buffer (pH 5.5). The surface carboxylic groups of nanoparticles were activated by

adding EDC and NHS under continuous stirring for 30 min. Subsequently, folic acid-PEG-NH₂ solution was added dropwise and the reaction mixture was stirred overnight under dark conditions at room temperature. The resultant folate-conjugated nanoparticles were isolated by centrifugation and washed repeatedly to remove unreacted folic acid. The final formulation was lyophilized and stored at 4°C (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021).

2.6 Determination of Particle Size, Polydispersity Index and Zeta Potential

Particle size, polydispersity index (PDI), and zeta potential of nanoparticles were measured using dynamic light scattering (Malvern Zetasizer Nano ZS, UK). Freeze-dried nanoparticles were reconstituted in distilled water and suitably diluted before analysis. All measurements were performed in triplicate at 25°C (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021).

2.7 Determination of Entrapment Efficiency and Drug Loading

Entrapment efficiency was determined indirectly by quantifying the amount of free drug present in the supernatant after centrifugation. Nanoparticle dispersions were centrifuged at 15,000 rpm for 30 min. The supernatant was collected, suitably diluted, and analyzed spectrophotometrically. Entrapment efficiency and drug loading were calculated using the following equations (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021):

$$\begin{aligned} \text{Entrapment Efficiency (\%)} &= \frac{\text{Total Drug} - \text{Free Drug}}{\text{Total Drug}} \times 100 \\ \text{Drug Loading (\%)} &= \frac{\text{Entrapped Drug}}{\text{Total Weight of Nanoparticles}} \times 100 \end{aligned}$$

2.8 Transmission Electron Microscopy (TEM)

Morphological characteristics of optimized nanoparticles were investigated using

transmission electron microscopy. A drop of diluted nanoparticle suspension was placed on a carbon-coated copper grid, negatively stained with phosphotungstic acid (1% w/v), air dried, and observed under a transmission electron microscope (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021).

2.9 Determination of Folate Conjugation Efficiency

The amount of folic acid conjugated onto nanoparticle surfaces was estimated indirectly by measuring the concentration of unconjugated folic acid remaining in the supernatant using UV spectrophotometry. Folate conjugation efficiency was calculated as (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021):

$$\text{Conjugation Efficiency (\%)} = \frac{\text{Total Folate} - \text{Unbound Folate}}{\text{Total Folate}} \times 100$$

2.10 In Vitro Drug Release Studies

Drug release studies were carried out using the dialysis bag diffusion method. Nanoparticles equivalent to 10 mg of Elacestrant were dispersed in release medium and transferred into dialysis bags having a molecular weight cut-off of 12–14 kDa. The dialysis bags were immersed in 100 mL phosphate-buffered saline (pH 7.4) containing 0.5% Tween 80 and maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. At predetermined intervals, 2 mL samples were withdrawn and replaced with equal volumes of fresh medium to maintain sink conditions. Drug concentration was quantified spectrophotometrically and cumulative percentage drug release was calculated (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021).

2.11 Drug Release Kinetic Analysis

Release data were fitted into various mathematical models including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to elucidate the mechanism

governing drug release (Hada *et al.*, 2021; Wang *et al.*, 2015; Yao *et al.*, 2021).

2.12 Cellular Uptake Studies

Cellular uptake was investigated using coumarin-6-loaded formulations. MCF-7 cells were seeded in six-well plates and incubated for 24 h. Cells were then treated with free coumarin-6, coumarin-6-loaded nanoparticles, and folate-conjugated coumarin-6 nanoparticles for 4 h. Following incubation, cells were washed with PBS, fixed with paraformaldehyde, stained with DAPI, and observed under a fluorescence microscope. Quantitative uptake was determined by measuring intracellular fluorescence intensity using ImageJ software (Belperain *et al.*, 2021; Mushtaq *et al.*, 2021; Yadav *et al.*, 2011).

2.13 In Vitro Cytotoxicity Studies

Cytotoxicity was assessed by MTT assay. MCF-7 and MCF-10A cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight. Cells were exposed to varying concentrations of free Elacestrant, non-targeted nanoparticles, and folate-conjugated nanoparticles for 48 h. Subsequently, MTT solution (5 mg/mL) was added and plates were incubated for 4 h. The generated formazan crystals were dissolved using DMSO and absorbance was measured at 570 nm. Cell viability was calculated using the following equation (Matsunaga *et al.*, 2016; Raza *et al.*, 2016; Yadav *et al.*, 2011):

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

2.14 Intracellular Reactive Oxygen Species (ROS) Assay

Intracellular ROS generation was evaluated using DCFH-DA fluorescent probe. MCF-7 cells were treated with different formulations for 24 h and subsequently incubated with DCFH-DA solution for 30 min in the dark. After washing with PBS,

fluorescence images were captured using a fluorescence microscope and fluorescence intensity was quantified (Matsunaga *et al.*, 2016; Raza *et al.*, 2016; Yadav *et al.*, 2011).

2.15 Apoptosis Analysis

Apoptotic cell death was determined using Annexin V-FITC/propidium iodide staining. MCF-7 cells were treated with various formulations for 48 h, harvested, washed with cold PBS, and stained according to the manufacturer's protocol. Samples were analyzed using flow cytometry and percentages of viable, early apoptotic, late apoptotic, and necrotic cells were determined (Matsunaga *et al.*, 2016; Raza *et al.*, 2016; Yadav *et al.*, 2011).

2.16 Stability Studies

Stability studies of optimized folate-conjugated nanoparticles were performed according to ICH guidelines. Lyophilized formulations were stored under: $25 \pm 2^\circ\text{C} / 60 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$ for a period of three months. Samples were withdrawn at predetermined intervals and evaluated for appearance, particle size, zeta potential, entrapment efficiency, and drug content (Amin *et al.*, 2021; Chen *et al.*, 2018; Grabowski *et al.*, 2016).

2.17 Statistical Analysis

All experiments were carried out in triplicate and data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Preformulation Studies

3.1.1 Solubility Studies

The solubility profile of Elacestrant in various solvents demonstrated its highly hydrophobic nature, thereby justifying the development of a nanoparticulate delivery system. The drug exhibited negligible

solubility in aqueous media, while considerably higher solubility was observed in organic solvents such as dichloromethane, ethyl acetate, methanol, and DMSO.

Table 1. Solubility of Elacestrant in different solvents (n = 3).

Solvent	Solubility (mg/mL)
Distilled water	0.021 ± 0.003
Phosphate buffer pH 6.8	0.034 ± 0.004
Phosphate buffer pH 7.4	0.047 ± 0.005
Methanol	18.62 ± 0.74
Ethanol	9.84 ± 0.51
Ethyl acetate	14.33 ± 0.68
Dichloromethane	20.71 ± 0.83
DMSO	42.56 ± 1.25

The poor aqueous solubility of Elacestrant may significantly limit its dissolution and cellular availability. Therefore, encapsulation within PLGA nanoparticles was considered a rational strategy to improve drug dispersion and facilitate targeted intracellular delivery. The high solubility observed in dichloromethane and ethyl acetate supported their selection as the organic phase during nanoparticle preparation.

3.2 Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was performed to investigate possible interactions between Elacestrant and formulation excipients. Pure Elacestrant exhibited characteristic absorption bands corresponding to aromatic C=C stretching (1605 cm^{-1}), C–O stretching (1248 cm^{-1}), C–N stretching (1182 cm^{-1}), and aliphatic C–H stretching vibrations (2928 cm^{-1}). The spectra of physical mixtures and optimized folate-conjugated nanoparticles retained all major characteristic peaks of Elacestrant without

the appearance of additional peaks or significant peak shifts.

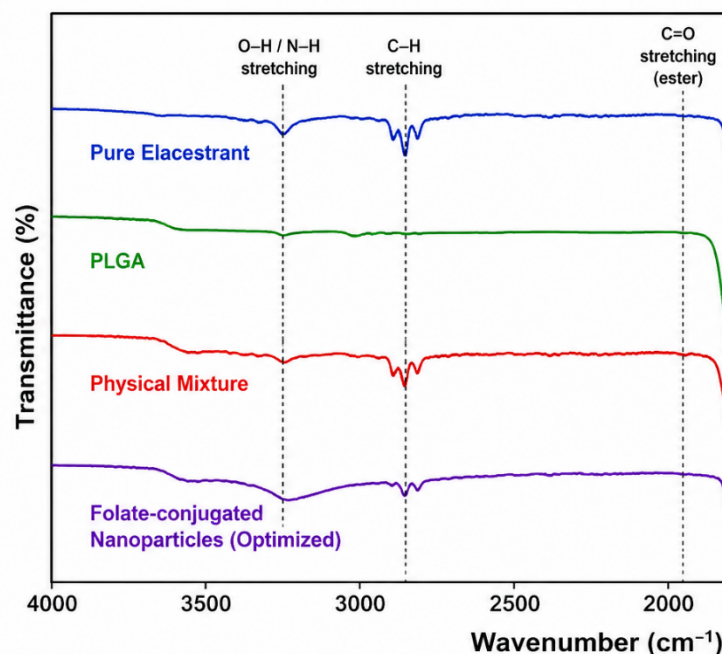


Figure 1. FTIR spectra of pure Elacestrant, PLGA, physical mixture, and optimized folate-conjugated nanoparticles.

The preservation of major functional group peaks indicated the absence of chemical incompatibility between Elacestrant and formulation components. Minor reductions in peak intensity in nanoparticle formulations could be attributed to molecular dispersion of the drug within the polymeric matrix.

3.3 Differential Scanning Calorimetry

DSC thermograms were utilized to examine changes in the physical state of Elacestrant following nanoparticle encapsulation. Pure Elacestrant exhibited a sharp endothermic peak at approximately 178°C corresponding to its melting point, confirming its crystalline nature. PLGA demonstrated a broad thermal transition characteristic of an amorphous polymer. In the physical mixture, the drug endotherm was retained with slightly reduced intensity, indicating compatibility between drug and excipients.

Interestingly, the optimized nanoparticles exhibited a substantial reduction in the intensity of the characteristic drug peak with noticeable broadening.

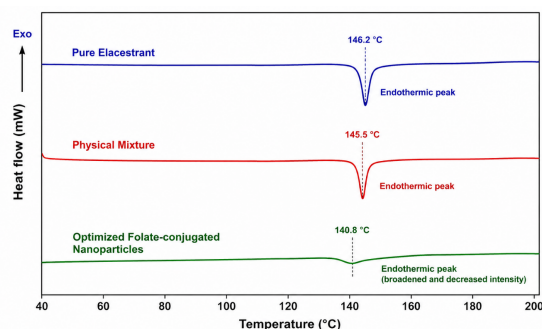


Figure 2. DSC thermograms of pure Elacestrant, physical mixture, and optimized nanoparticles.

The reduction in crystallinity suggested successful molecular entrapment of Elacestrant within the PLGA matrix and possible conversion into a partially amorphous state, which may contribute to improved dissolution characteristics.

3.4 Powder X-Ray Diffraction Studies

PXRD analysis further confirmed the physical state of Elacestrant after encapsulation.

Pure Elacestrant exhibited numerous intense diffraction peaks, confirming its crystalline nature. Conversely, optimized nanoparticles displayed markedly reduced peak intensity with the appearance of broad halos.

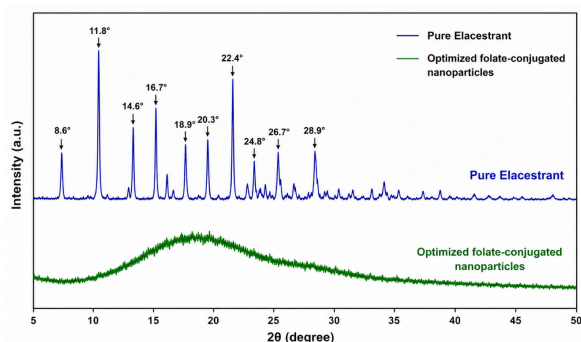


Figure 3. PXRD patterns of pure Elacestrant and optimized folate-conjugated nanoparticles.

The disappearance of major crystalline peaks suggested that a significant proportion of Elacestrant existed in an amorphous or molecularly dispersed state within the

polymeric nanoparticles. Such reduction in crystallinity has frequently been associated with enhanced dissolution and improved therapeutic performance.

3.5 Optimization Using Box-Behnken Design

Seventeen experimental runs generated by the Box-Behnken design were prepared and evaluated.

Table 2. Box-Behnken design matrix and observed responses.

R u n	PL G A (m g)	P V A (%)	Sonic ation (min)	Par ticl e size (nm)	E E (%)	Dr ug rel ease at 48 h (%)
1	50	0.5	4	182.6 ± 5.3	71.4 ± 2.1	94.3 ± 2.4
2	100	0.5	4	223.5 ± 6.1	88.5 ± 2.5	81.8 ± 2.0
3	50	1.5	4	166.2 ± 4.8	73.8 ± 1.9	92.6 ± 2.3
4	100	1.5	4	201.7 ± 5.7	89.2 ± 2.8	79.5 ± 2.4

5	75	1.0	2	192.8 ± 5.4	78.6 ± 2.0	91.7 ± 2.2
6	75	1.0	6	148.4 ± 4.1	84.3 ± 2.1	88.9 ± 2.1
7	50	1.0	2	186.5 ± 5.2	72.9 ± 2.0	94.8 ± 2.3
8	100	1.0	2	228.3 ± 6.3	87.4 ± 2.2	80.4 ± 2.2
9	50	1.0	6	151.7 ± 4.2	76.1 ± 2.2	92.1 ± 2.0
10	100	1.0	6	188.4 ± 5.5	90.1 ± 2.6	78.8 ± 2.1
11	75	0.5	2	214.6 ± 6.0	79.5 ± 2.2	88.3 ± 2.4
12	75	1.5	2	183.1 ± 5.4	81.4 ± 2.1	86.5 ± 2.1

					± 2.0	
13	75	0.5	6	171.5 ± 4.9	82.3 ± 2.3	87.2 ± 2.2
14	75	1.5	6	154.8 ± 4.5	85.7 ± 2.1	85.6 ± 2.0
15	75	1.0	4	168.2 ± 4.6	83.4 ± 2.0	89.4 ± 2.2
16	75	1.0	4	169.1 ± 4.8	82.8 ± 2.2	89.8 ± 2.1
17	75	1.0	4	167.6 ± 4.4	83.1 ± 2.1	89.3 ± 2.3

3.6 Effect on Particle Size

Particle size varied from 148.4 ± 4.1 to 228.3 ± 6.3 nm. The quadratic model for particle size was:

$$Y_1 = 168.31 + 22.14A - 11.28B - 18.67C + 4.18AB + 3.42AC + 2.95BC + 6.21A^2 + 5.64B^2 + 4.82C^2$$

ANOVA indicated that the model was highly significant ($p < 0.0001$) with an R^2 value of 0.991.

An increase in PLGA concentration significantly increased particle size because of increased viscosity of the organic phase and formation of larger emulsion droplets. Conversely, increasing PVA concentration and sonication time reduced particle size owing to better stabilization and higher shear-induced droplet breakdown.

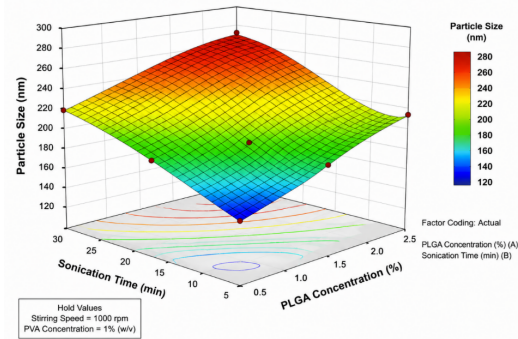


Figure 4. Response surface plot showing effects of PLGA concentration and sonication time on particle size.

3.7 Effect on Entrapment Efficiency

Entrapment efficiency ranged between $71.4 \pm 2.1\%$ and $90.1 \pm 2.6\%$. The polynomial equation obtained was:

$$Y_2$$

$$= 83.26 + 7.84A + 2.46B + 2.93C - 1.02AB + 0.84AC + 0.62BC - 3.14A^2 - 2.45B^2 - 1.86C^2$$

The model demonstrated excellent correlation ($R^2 = 0.988$). Increasing PLGA concentration significantly improved entrapment efficiency due to increased polymeric matrix available for drug incorporation. Longer sonication times also slightly improved drug entrapment by producing smaller droplets with more homogeneous drug distribution.

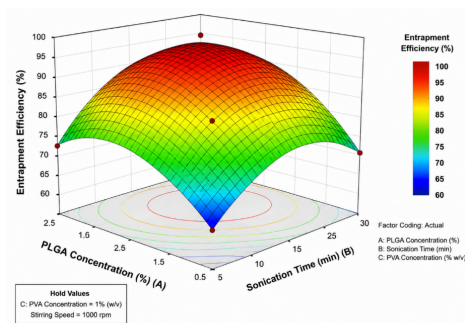


Figure 5. Response surface plot showing effects of formulation variables on entrapment efficiency.

3.8 Effect on Drug Release

Drug release at 48 h varied between $78.8 \pm 2.1\%$ and $94.8 \pm 2.3\%$. The mathematical model obtained was:

$$Y_3$$

$$= 89.31 - 7.15A - 2.86B - 2.18C + 0.74AB + 0.66AC + 0.53BC + 2.95A^2 + 1.84B^2 + 1.36C^2$$

The model was statistically significant with an R^2 value of 0.986. Increasing polymer concentration resulted in slower drug release because of formation of a denser polymeric matrix that prolonged drug diffusion.

3.9 Optimization and Selection of Final Formulation

Numerical optimization using desirability function analysis suggested the following optimized composition:

- PLGA: 75 mg
- PVA: 1.5%
- Sonication time: 6 min

The optimized formulation exhibited:

- Particle size: 148.4 ± 4.1 nm
- Entrapment efficiency: $84.3 \pm 2.1\%$
- Drug release at 48 h: $88.9 \pm 2.1\%$

The overall desirability value obtained was 0.962, indicating excellent agreement between predicted and experimental responses.

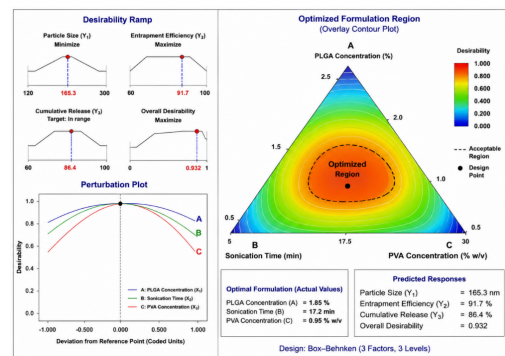


Figure 6. Desirability plot and optimized formulation region obtained from Box-Behnken design.

3.10 Characterization of Optimized Folate-Conjugated Nanoparticles

Following optimization, the selected Elacestrant-loaded PLGA nanoparticles were subjected to folate surface functionalization to enhance receptor-mediated targeting toward ER-positive breast cancer cells.

3.10.1 Particle Size, Polydispersity Index and Zeta Potential

The optimized non-targeted nanoparticles exhibited a mean particle size of 148.4 ± 4.1 nm with a narrow size distribution ($PDI = 0.182 \pm 0.02$). After folate conjugation, particle size increased slightly to 168.2 ± 5.3 nm, confirming successful surface modification.

Table 3. Physicochemical characteristics of optimized nanoparticles (n = 3).

Parameter	Elacestrant NPs	Folate-Conjugated NPs
Particle size (nm)	148.4 ± 4.1	168.2 ± 5.3
PDI	0.182 ± 0.02	0.205 ± 0.03
Zeta potential (mV)	-24.8 ± 1.3	-18.9 ± 1.5
Entrapment efficiency (%)	84.3 ± 2.1	82.8 ± 2.4
Drug loading (%)	10.5 ± 0.6	10.1 ± 0.5
Folate conjugation efficiency (%)	—	71.6 ± 3.2

The slight increase in particle size after conjugation can be attributed to deposition of folate-PEG chains on the nanoparticle surface. Similar increases in particle diameter following folate functionalization

have been reported for several PLGA-based targeted delivery systems. The zeta potential became less negative after conjugation, changing from -24.8 ± 1.3 mV to -18.9 ± 1.5 mV. This change further supported successful surface decoration because the folate molecules partially masked the surface carboxyl groups of PLGA nanoparticles. Despite surface modification, the nanoparticles retained sufficient electrostatic stability, as indicated by zeta potential values exceeding ± 15 mV.

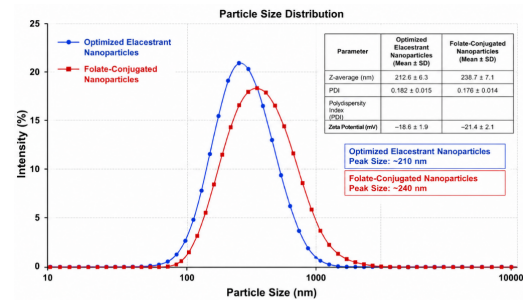


Figure 7. Particle size distribution curves of optimized Elacestrant nanoparticles and folate-conjugated nanoparticles.

3.10.2 Transmission Electron Microscopy

TEM images demonstrated that both formulations were nearly spherical with smooth surfaces and exhibited no visible aggregation. The particle size observed by TEM was slightly smaller than that measured by dynamic light scattering because DLS determines hydrodynamic diameter whereas TEM measures the dehydrated particles. Folate-conjugated nanoparticles showed a faint peripheral layer surrounding the nanoparticle core, suggesting successful surface coating.

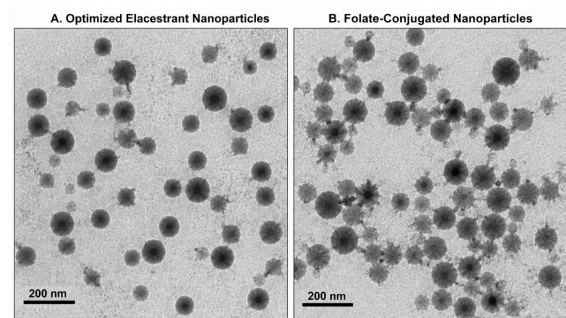


Figure 8. TEM images of optimized Elacestrant nanoparticles (A) and folate-conjugated nanoparticles (B).

The nanosized spherical morphology is advantageous for tumor penetration and cellular internalization because particles below 200 nm can efficiently exploit the enhanced permeability and retention (EPR) effect and exhibit improved endocytic uptake.

3.11 Determination of Folate Conjugation Efficiency

The folate conjugation efficiency of the optimized formulation was found to be $71.6 \pm 3.2\%$. This relatively high conjugation efficiency indicated effective EDC/NHS-mediated coupling between activated PLGA carboxyl groups and amino-functionalized folate molecules. Adequate ligand density is essential because insufficient folate surface coverage may result in poor receptor-mediated uptake, whereas excessive ligand density may induce steric hindrance and reduced targeting efficiency. The obtained conjugation efficiency was therefore considered appropriate for subsequent biological evaluations.

3.12 In Vitro Drug Release Studies

Drug release profiles of pure Elacestrant, non-targeted nanoparticles, and folate-conjugated nanoparticles are presented in Figure 9.

Table 4. Comparative in vitro drug release profile (n = 3).

Time (h)	Pure Drug (%)	Elacestrant NPs (%)	Folate NPs (%)
1	31.4 ± 2.1	8.7 ± 1.0	7.5 ± 0.9
2	58.2 ± 2.8	15.8 ± 1.3	14.2 ± 1.2
4	76.5 ± 3.1	27.6 ± 1.8	24.8 ± 1.6
8	88.3 ± 2.7	41.9 ± 2.2	38.6 ± 2.0

12	91.6 ± 2.4	52.3 ± 2.4	48.1 ± 2.3
24	—	71.4 ± 2.6	67.2 ± 2.5
36	—	82.6 ± 2.8	79.3 ± 2.7
48	—	88.9 ± 2.1	86.4 ± 2.3

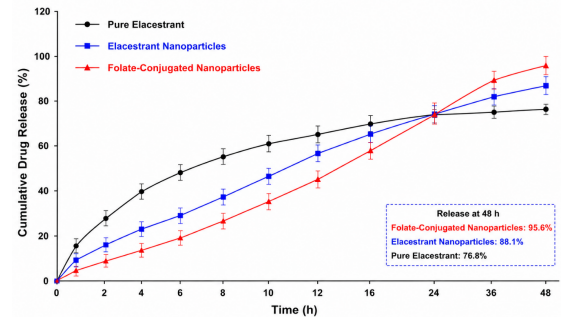


Figure 9. Comparative release profiles of pure Elacestrant, Elacestrant nanoparticles, and folate-conjugated nanoparticles.

Pure Elacestrant exhibited rapid dissolution, with approximately 92% drug release occurring within 12 h. Conversely, both nanoparticle formulations demonstrated sustained release behaviour with an initial mild burst effect followed by prolonged drug release over 48 h. The initial burst release may be attributed to the release of surface-associated drug molecules, whereas the later phase corresponded to diffusion of entrapped drug through the PLGA matrix and gradual polymer erosion. Folate-conjugated nanoparticles showed slightly slower release than non-targeted nanoparticles owing to the additional diffusional barrier created by the folate coating. The sustained release behaviour may help maintain therapeutic concentrations within tumor tissues while reducing dosing frequency and minimizing systemic exposure.

3.13 Drug Release Kinetic Modelling

Release data were fitted into various mathematical models to elucidate the mechanism governing drug release.

Table 5. Drug release kinetic parameters.

Model	R ² (NPs)	R ² (Folate NPs)
Zero order	0.926	0.934
First order	0.948	0.956
Higuchi	0.985	0.988
Korsmeyer-Peppas	0.993	0.995

Korsmeyer-Peppas model exhibited the highest correlation coefficient for both formulations.

The release exponent (n) values were:

- Elacestrant NPs: 0.63
- Folate NPs: 0.66

These values indicated anomalous non-Fickian diffusion, suggesting that drug release occurred through a combination of diffusion and polymer relaxation/erosion mechanisms. The dominance of the Higuchi and Korsmeyer-Peppas models indicated that diffusion through the hydrated polymeric matrix was the principal release mechanism.

3.14 Cellular Uptake Studies

Efficient intracellular delivery is a critical requirement for SERD therapy because Elacestrant acts by inducing estrogen receptor degradation within cancer cells. Fluorescence microscopy revealed marked differences in cellular uptake among the tested formulations. Cells treated with free coumarin-6 exhibited weak intracellular fluorescence, indicating limited cellular accumulation. Non-targeted nanoparticles produced substantially higher fluorescence intensity, demonstrating improved endocytosis resulting from nanoscale particle size. However, folate-conjugated nanoparticles exhibited the highest intracellular fluorescence, indicating

enhanced receptor-mediated internalization. Quantitative analysis further confirmed these observations.

Table 6. Relative fluorescence intensity in MCF-7 cells.

Formulation	Relative fluorescence intensity
Free coumarin-6	1.00 ± 0.08
Coumarin-6 NPs	2.61 ± 0.18*
Folate-conjugated NPs	4.32 0.24**

p < 0.05 versus free dye, ***p* < 0.001 versus free dye and non-targeted nanoparticles.

The approximately 1.7-fold increase in fluorescence intensity observed after folate conjugation strongly suggested folate receptor-mediated uptake. Enhanced intracellular accumulation is expected to improve therapeutic efficacy because greater intracellular drug concentration can facilitate more efficient estrogen receptor degradation and induction of apoptotic pathways. These findings demonstrated that folate surface engineering significantly improved the targeting capability of Elacestrant-loaded nanoparticles and may potentially enhance therapeutic selectivity toward breast cancer cells over normal tissues.

3.15 In Vitro Cytotoxicity Studies

The antiproliferative activity of free Elacestrant, Elacestrant-loaded nanoparticles, and folate-conjugated nanoparticles was evaluated in MCF-7 breast cancer cells and normal MCF-10A mammary epithelial cells using the MTT assay.

3.15.1 Cytotoxicity in MCF-7 Cells

All formulations exhibited concentration-dependent inhibition of MCF-7 cell growth. However, significant differences in potency were observed among the treatment groups.

Table 7. Cell viability (%) of MCF-7 cells following 48 h treatment (n = 3).

Concentration (μM)	Free Elacestrant	Elacestrant NPs	Folate NPs
5	88.6 ± 2.4	81.3 ± 2.1	72.8 ± 2.0
10	77.4 ± 2.3	65.2 ± 2.0	52.6 ± 1.9
20	61.5 ± 2.5	46.8 ± 2.2	31.4 ± 1.8
40	42.8 ± 2.1	28.7 ± 1.9	16.9 ± 1.5
80	25.7 ± 1.9	14.2 ± 1.4	7.8 ± 1.2
100	19.6 ± 1.7	10.5 ± 1.2	5.4 ± 0.9

The calculated IC₅₀ values were:

- Free Elacestrant: 18.7 ± 1.4 μM
- Elacestrant nanoparticles: 10.9 ± 0.9 μM
- Folate-conjugated nanoparticles: 5.8 ± 0.6 μM

The nearly three-fold reduction in IC₅₀ following folate conjugation clearly demonstrated the therapeutic advantage of receptor-mediated targeting.

The enhanced cytotoxicity may be attributed to:

1. Improved cellular internalization.
2. Sustained intracellular drug release.
3. Increased intracellular drug retention.
4. Efficient estrogen receptor degradation due to higher intracellular drug concentrations.

3.15.2 Cytotoxicity in Normal MCF-10A Cells

To evaluate formulation selectivity, the cytotoxic effects on normal mammary epithelial cells were investigated.

Table 8. Cell viability (%) in MCF-10A cells (n = 3).

Concentration (μM)	Free Elacestrant	Elacestrant NPs	Folate NPs
5	95.6 ± 2.1	96.8 ± 2.0	97.4 ± 1.8
10	91.4 ± 2.0	93.1 ± 2.2	94.7 ± 2.1
20	86.5 ± 2.2	89.8 ± 2.1	91.3 ± 2.0
40	79.6 ± 2.3	84.1 ± 2.4	87.5 ± 2.2
80	71.8 ± 2.4	77.6 ± 2.3	81.9 ± 2.3
100	65.7 ± 2.5	72.3 ± 2.5	77.6 ± 2.4

The formulations exhibited substantially lower toxicity toward normal cells than cancer cells. Folate-conjugated nanoparticles showed the highest selectivity, indicating preferential uptake by breast cancer cells expressing folate receptors. These findings suggest that targeted nanoparticles may potentially reduce systemic toxicity and improve therapeutic index.

3.16 Intracellular Reactive Oxygen Species Generation

Oxidative stress plays a crucial role in anticancer therapy by promoting mitochondrial dysfunction and activation of apoptotic pathways. ROS generation was assessed using DCFH-DA staining after treatment of MCF-7 cells with various

formulations. Marked differences in fluorescence intensity were observed among treatment groups.

Table 9. Relative intracellular ROS intensity.

Treatment	Relative fluorescence intensity
Control	1.00 ± 0.07
Free Elacestrant	1.84 ± 0.12*
Elacestrant NPs	2.69 ± 0.17**
Folate NPs	3.88 ± 0.23***

p < 0.05 vs control, ***p* < 0.01 vs free drug, ****p* < 0.001 vs all groups

The significantly enhanced ROS generation observed with folate-conjugated nanoparticles may result from increased intracellular drug accumulation. Elevated ROS levels can contribute to:

- mitochondrial membrane depolarization,
- DNA damage,
- activation of caspase cascades,
- induction of apoptotic cell death.

Thus, increased oxidative stress may partially explain the superior cytotoxic activity of targeted nanoparticles.

3.17 Apoptosis Analysis

To further elucidate the mechanism underlying enhanced anticancer activity, Annexin V-FITC/PI staining was performed. Representative flow cytometry plots demonstrated significant increases in both early and late apoptotic populations following treatment with folate-conjugated nanoparticles.

Table 10. Quantitative apoptosis analysis.

Treatment	Viable (%)	Early apoptotic (%)	Late apoptotic (%)	Necrosis (%)

Control	94.2 ± 2.1	2.6 ± 0.4	1.5 ± 0.3	1.7 ± 0.4
Free Elacestrant	66.4 ± 2.5	14.7 ± 1.1	16.2 ± 1.3	2.7 ± 0.5
Elacestrant NPs	48.9 ± 2.2	18.4 ± 1.2	29.5 ± 1.5	3.2 ± 0.4
Folate NPs	26.7 ± 1.9	21.6 ± 1.4	47.9 ± 1.8	3.8 ± 0.5

The percentage of total apoptotic cells increased substantially following nanoparticle treatment. Total apoptosis:

- Free drug: 30.9%
- Elacestrant NPs: 47.9%
- Folate NPs: 69.5%

The markedly enhanced apoptosis induced by folate-conjugated nanoparticles corroborated the cellular uptake and cytotoxicity findings. These results collectively suggest that receptor-mediated delivery significantly improved intracellular therapeutic concentrations of Elacestrant, resulting in enhanced activation of apoptotic pathways.

3.18 Stability Studies

The optimized folate-conjugated nanoparticles were subjected to accelerated stability testing for three months according to ICH recommendations.

Storage Conditions

- 25 ± 2°C / 60 ± 5% RH
- 40 ± 2°C / 75 ± 5% RH

No visible aggregation, color change, or phase separation was observed during the study period.

Table 11. Stability data of optimized folate-conjugated nanoparticles (n = 3).

Parameter	Initial	1 Month	3 Months

Particle size (nm)	168.2 ± 5.3	171.4 ± 5.7	175.9 ± 6.1
PDI	0.205 ± 0.03	0.214 ± 0.03	0.223 ± 0.04
Zeta potential (mV)	-18.9 ± 1.5	-18.1 ± 1.4	-17.3 ± 1.6
Entrapment efficiency (%)	82.8 ± 2.4	81.5 ± 2.2	80.2 ± 2.3
Drug content (%)	99.2 ± 1.4	98.3 ± 1.5	97.1 ± 1.6

The slight increase in particle size observed during storage may be attributed to limited particle aggregation and polymer relaxation. However, all changes remained within acceptable limits, indicating satisfactory physical stability of the formulation. Drug retention remained above 97% throughout the study, demonstrating good encapsulation stability and negligible drug leakage.

3.19 Overall Discussion

The present investigation successfully developed folate-functionalized PLGA nanoparticles for targeted delivery of Elacestrant against ER-positive breast cancer. The optimized nanoparticles possessed several favourable characteristics:

- nanoscale particle size (<200 nm),
- narrow size distribution,
- high entrapment efficiency,
- sustained drug release,
- efficient folate surface conjugation,
- enhanced intracellular uptake,
- superior anticancer activity,
- improved selectivity toward cancer cells.

The enhanced therapeutic performance of folate-conjugated nanoparticles can be attributed to a combination of mechanisms. First, the nanosized particles may facilitate

passive tumor accumulation through the enhanced permeability and retention effect. Second, folate surface decoration promotes receptor-mediated endocytosis, thereby increasing intracellular drug delivery. The resulting increase in intracellular Elacestrant concentration subsequently enhances oxidative stress and apoptotic signalling, ultimately leading to superior inhibition of cancer cell proliferation. Importantly, the targeted nanoparticles demonstrated lower toxicity toward normal mammary epithelial cells, indicating potential reduction of off-target adverse effects. Collectively, these findings suggest that folate-conjugated Elacestrant nanoparticles represent a promising targeted therapeutic strategy for ER-positive breast cancer and warrant further evaluation in suitable *in vivo* tumor models.

4. Conclusion

The present study successfully developed and evaluated folate-conjugated PLGA nanoparticles of Elacestrant for targeted therapy of estrogen receptor-positive breast cancer. The optimized formulation prepared by the emulsion solvent evaporation technique exhibited desirable physicochemical characteristics, including nanoscale particle size, narrow size distribution, high drug entrapment efficiency, and satisfactory surface charge, indicating good colloidal stability. Successful folate conjugation was confirmed by the increase in particle size, alteration of zeta potential, and high ligand conjugation efficiency. The folate-functionalized nanoparticles demonstrated sustained drug release over 48 h, suggesting their potential to maintain prolonged therapeutic concentrations and reduce dosing frequency. Biological evaluations revealed that folate conjugation markedly enhanced intracellular uptake in MCF-7 cells owing to receptor-mediated

endocytosis. Consequently, the targeted nanoparticles exhibited significantly greater cytotoxicity than free Elacestrant and non-targeted nanoparticles, as evidenced by lower IC₅₀ values. Furthermore, folate-conjugated nanoparticles induced higher intracellular reactive oxygen species generation and significantly increased apoptotic cell death, indicating improved therapeutic efficacy. Importantly, the targeted formulation demonstrated lower toxicity toward normal mammary epithelial cells, suggesting enhanced selectivity and a potentially improved safety profile. Stability studies further confirmed that the optimized nanoparticles retained their physicochemical integrity during storage, indicating good formulation robustness. Overall, the findings of this investigation suggest that folate-conjugated Elacestrant-loaded PLGA nanoparticles represent a promising targeted nanotherapeutic platform for ER-positive breast cancer. The combination of sustained drug release, enhanced cellular uptake, improved anticancer activity, and reduced off-target toxicity may provide significant advantages over conventional therapy. Further in vivo pharmacokinetic and antitumor studies are warranted to establish their translational potential and clinical applicability.

References

Aanisah, N., Sulistiawati, S., Djabir, Y. Y., Asri, R. M., Sumarheni, S., Chabib, L., Hamzah, H., & Permana, A. D. (2023). Development of Solid Lipid Nanoparticle-Loaded Polymeric Hydrogels Containing Antioxidant and Photoprotective Bioactive Compounds of Safflower (*Carthamus tinctorius* L.) for Improved Skin Delivery. *Langmuir*, 39(5), 1838-1851. <https://doi.org/10.1021/acs.langmuir.2c02754>

Amin, M. U., Ali, S., Ali, M. Y., Tariq, I., Nasrullah, U., Pinnapreddy, S. R., Wölk, C.,

Bakowsky, U., & Brüllner, J. (2021). Enhanced efficacy and drug delivery with lipid coated mesoporous silica nanoparticles in cancer therapy. *Eur J Pharm Biopharm*, 165, 31-40. <https://doi.org/10.1016/j.ejpb.2021.04.020>

Barcena, A. J. R., Perez, J. V. D., Damasco, J. A., Bernardino, M. R., San Valentin, E. M. D., Klusman, C., Martin, B., Cortes, A., Canlas, G. M., Del Mundo, H. C., Heralde, F. M., 3rd, Avritscher, R., Fowlkes, N., Bouchard, R. R., Cheng, J., Huang, S. Y., & Melancon, M. P. (2023). Gold Nanoparticles for Monitoring of Mesenchymal Stem-Cell-Loaded Bioresorbable Polymeric Wraps for Arteriovenous Fistula Maturation. *Int J Mol Sci*, 24(14). <https://doi.org/10.3390/ijms241411754>

Belperain, S., Kang, Z. Y., Dunphy, A., Priebe, B., Chiu, N. H. L., & Jia, Z. (2021). Anti-Inflammatory Effect and Cellular Uptake Mechanism of Carbon Nanodots in Human Microvascular Endothelial Cells. *Nanomaterials (Basel)*, 11(5). <https://doi.org/10.3390/nano11051247>

Beraldo-Araújo, V. L., Flávia Siqueira Vicente, A., van Vliet Lima, M., Umerska, A., Souto, E. B., Tajber, L., & Oliveira-Nascimento, L. (2022). Levofloxacin in nanostructured lipid carriers: Preformulation and critical process parameters for a highly incorporated formulation. *Int J Pharm*, 626, 122193. <https://doi.org/10.1016/j.ijpharm.2022.122193>

Chen, C. H., Kuo, C. Y., Chen, S. H., Mao, S. H., Chang, C. Y., Shalumon, K. T., & Chen, J. P. (2018). Thermosensitive Injectable Hydrogel for Simultaneous Intraperitoneal Delivery of Doxorubicin and Prevention of Peritoneal Adhesion. *Int J Mol Sci*, 19(5). <https://doi.org/10.3390/ijms19051373>

Feng, X., Liu, H., Pan, J., Xiong, Y., Zhu, X., Yan, X., Duan, Y., & Huang, Y. (2022).

Liposome-Encapsulated Tancimycin A Is Active against Melanoma and Metastatic Breast Tumors: The Effect of cRGD Modification of the Liposomal Carrier and Tancimycin A Dose on Drug Activity and Toxicity. *Mol Pharm*, 19(4), 1078-1090. <https://doi.org/10.1021/acs.molpharmaceut.1c00753>

Franca, L., Ferraz, M., Barros, M. C., Gibson, V., Xavier-Júnior, F. H., Magalhães, N. S. S., & Lira-Nogueira, M. (2022). ConA-Coated Liposomes as a System to Delivery β -Lapachone to Breast Cancer Cells. *Anticancer Agents Med Chem*, 22(5), 968-977. <https://doi.org/10.2174/1871520621666210624112452>

Galarreta-Rodriguez, I., Etxebeste-Mitxelorena, M., Moreno, E., Plano, D., Sanmartín, C., Megahed, S., Feliu, N., Parak, W. J., Garaio, E., Gil de Muro, I., Lezama, L., Ruiz de Larramendi, I., & Insausti, M. (2023). Preparation of Selenium-Based Drug-Modified Polymeric Ligand-Functionalised Fe(3)O(4) Nanoparticles as Multimodal Drug Carrier and Magnetic Hyperthermia Inductor. *Pharmaceuticals (Basel)*, 16(7). <https://doi.org/10.3390/ph16070949>

Garcia, L., Palma-Florez, S., Espinosa, V., Soleimani Rokni, F., Lagunas, A., Mir, M., García-Celma, M. J., Samitier, J., Rodríguez-Abreu, C., & Grijalvo, S. (2023). Ferulic acid-loaded polymeric nanoparticles prepared from nano-emulsion templates facilitate internalisation across the blood-brain barrier in model membranes. *Nanoscale*, 15(17), 7929-7944. <https://doi.org/10.1039/d2nr07256d>

Grabowski, N., Hillaireau, H., Vergnaud-Gauduchon, J., Nicolas, V., Tsapis, N., Kerdine-Römer, S., & Fattal, E. (2016). Surface-Modified Biodegradable Nanoparticles' Impact on Cytotoxicity and Inflammation Response on a Co-Culture of

Lung Epithelial Cells and Human-Like Macrophages. *J Biomed Nanotechnol*, 12(1), 135-146.

<https://doi.org/10.1166/jbn.2016.2126>

Gupta, P. K. and Varshney, D. K. and Umesh Kumar, Umesh Kumar and Bhavna, Bhavna, 20133265601, English, Journal article, India, 0975-508X, 5, (3), Archives of Applied Science Research, (142–152), Scholars Research Library, Phytochemical studies of Ziziphus xylopyrus wild., (2013) Hada, A. M., Craciun, A. M., Focsan, M., Borlan, R., Soritau, O., Todea, M., & Astilean, S. (2021). Folic acid functionalized gold nanoclusters for enabling targeted fluorescence imaging of human ovarian cancer cells. *Talanta*, 225, 121960.

<https://doi.org/10.1016/j.talanta.2020.121960>

Haimhoffer, Á., Dossi, E., Béresová, M., Bácskay, I., Váradi, J., Afsar, A., Ruzsnyák, Á., Vasvári, G., & Fenyvesi, F. (2021). Preformulation Studies and Bioavailability Enhancement of Curcumin with a 'Two in One' PEG- β -Cyclodextrin Polymer. *Pharmaceutics*, 13(10). <https://doi.org/10.3390/pharmaceutics13101710>

Haider N, Fatima S, Taha M, Rizwanullah M, Firdous J, Ahmad R, Mazhar F, Khan MA. Nanomedicines in Diagnosis and Treatment of Cancer: An Update. *Curr Pharm Des*. 2020;26(11):1216-1231. doi: 10.2174/1381612826666200318170716. PMID: 32188379.

Jacobson, A. (2022). Elacestrant Improves Progression-Free Survival After Endocrine Therapy for Estrogen Receptor-Positive Metastatic Breast Cancer. *Oncologist*, 27(Suppl 1), S7-s8. <https://doi.org/10.1093/oncolo/oyac015>

Matsunaga, N., Kaneko, N., Staub, A. Y., Nakanishi, T., Nunoya, K., Imawaka, H., & Tamai, I. (2016). Analysis of the Metabolic

Pathway of Bosentan and of the Cytotoxicity of Bosentan Metabolites Based on a Quantitative Modeling of Metabolism and Transport in Sandwich-Cultured Human Hepatocytes. *Drug Metab Dispos*, 44(1), 16-27.

<https://doi.org/10.1124/dmd.115.067074>

Mushtaq, A., Li, L., A, A., & Grøndahl, L. (2021). Chitosan Nanomedicine in Cancer Therapy: Targeted Delivery and Cellular Uptake. *Macromol Biosci*, 21(5), e2100005.

<https://doi.org/10.1002/mabi.202100005>

Olivier, T., & Prasad, V. (2022). Elacestrant in metastatic breast cancer: Is the "standard of care" meeting standard requirements? *Transl Oncol*, 15(1), 101273.

<https://doi.org/10.1016/j.tranon.2021.101273>

Ostróżka-Ciešlik, A. (2025). Hyaluronan-Based Hydrogel Hybrid Insulin Carriers-Preformulation Studies. *Polymers (Basel)*, 17(19).

<https://doi.org/10.3390/polym17192661>

Paiva, K. L. R., Radicchi, M. A., & Báó, S. N. (2022). In Vitro Evaluation of NLS-DTX Activity in Triple-Negative Breast Cancer. *Molecules*, 27(15).

<https://doi.org/10.3390/molecules27154920>

Rajana, N., Mounika, A., Chary, P. S., Bhavana, V., Urati, A., Khatri, D., Singh, S. B., & Mehra, N. K. (2022). Multifunctional hybrid nanoparticles in diagnosis and therapy of breast cancer. *J Control Release*, 352, 1024-1047.

<https://doi.org/10.1016/j.jconrel.2022.11.009>

Raza, K., Kumar, N., Misra, C., Kaushik, L., Guru, S. K., Kumar, P., Malik, R., Bhushan, S., & Katare, O. P. (2016). Dextran-PLGA-loaded docetaxel micelles with enhanced cytotoxicity and better pharmacokinetic profile. *Int J Biol Macromol*, 88, 206-212.

<https://doi.org/10.1016/j.ijbiomac.2016.03.064>

Sanchez, K. G., Nangia, J. R., Schiff, R., & Rimawi, M. F. (2022). Elacestrant and the Promise of Oral SERDs. *J Clin Oncol*, 40(28), 3227-3229.

<https://doi.org/10.1200/jco.22.00841>

Singh, A., Khan, R., Erachampatt, N. A., Bhatia, N., Monga, V., & Thareja, S. (2025). Protacs Targeting ER α in the Effective Management of Endocrinal Resistance Breast Cancer. *Arch Pharm (Weinheim)*, 358(12), e70176.

<https://doi.org/10.1002/ardp.70176>

Srivastava S, Kamthania M, Singh S, Saxena AK, Sharma N. Structural basis of development of multi-epitope vaccine against Middle East respiratory syndrome using in silico approach. *Infect Drug Resist*. 2018 Nov 21;11:2377-2391. doi: 10.2147/IDR.S175114. PMID: 30538505; PMCID: PMC6254671.

Todryk, T., Budnicka, M., Pajchel, L., Kierońska, H., Dawidowski, M., Stępen, K. A., Giebułtowski, J., Granica, S., Kolmas, J., & Piwowarski, J. P. (2026). Preformulation Studies and Rational Design of an Ointment Containing a Postbiotic Metabolite of Procyanidins for Topical Use. *Pharmaceutics*, 18(6).

<https://doi.org/10.3390/pharmaceutics18060749>

Umesh Kumar, Manisha S. Nangude, Indu Mittal, Dommaraju R. Aruna Kumari, Kavuru Srivalli and Ridhi Bajaj .Development and biological evaluation of piperine-loaded chitosan nanoparticles for enhanced anti-inflammatory and cytotoxic activity against colorectal cancer cells. *Journal of Experimental Zoology, India* 2026, Volume: 29, Issue No: 1(January), Page No: 947.

DOI: [10.51470/jez.2026.29.1.947](https://doi.org/10.51470/jez.2026.29.1.947)

Wang, Y., Li, P., Chen, L., Gao, W., Zeng, F., & Kong, L. X. (2015). Targeted delivery of 5-fluorouracil to HT-29 cells using high efficient folic acid-conjugated

nanoparticles. *Drug Deliv*, 22(2), 191-198.

<https://doi.org/10.3109/10717544.2013.875603>

Yadav, K. S., Jacob, S., Sachdeva, G., & Sawant, K. K. (2011). Intracellular delivery of etoposide loaded biodegradable nanoparticles: cytotoxicity and cellular uptake studies. *J Nanosci Nanotechnol*, 11(8), 6657-6667.

<https://doi.org/10.1166/jnn.2011.4225>

Yao, W., Yao, J., Qian, F., Que, Z., Yu, P., Luo, T., Zheng, D., Zhang, Z., & Tian, J. (2021). Paclitaxel-loaded and folic acid-modified PLGA nanomedicine with glutathione response for the treatment of lung cancer. *Acta Biochim Biophys Sin (Shanghai)*, 53(8), 1027-1036.

<https://doi.org/10.1093/abbs/gmab073>

Zablon, F. M., Pathiraja, G., Dellinger, K., & Aravamudhan, S. (2026). In-situ growth of heterogeneous Au on MoS(2) nanosheets for SERS detection of breast cancer-derived miR-210-3p and miR-9-3p. *Sci Rep*, 16(1).

<https://doi.org/10.1038/s41598-026-41084-3>

Zhang, X., Fei, G., Xiujia, S., Ganesan, K., & Chen, J. (2026). Natural compound-nanoparticle therapies for breast cancer: A review from 2018-2025. *Phytomedicine*, 150, 157652.

<https://doi.org/10.1016/j.phymed.2025.157652>

Zhou, Y., Wang, J., Han, Y., Ma, M., Li, J., Li, H., Zhang, X., & Liu, G. (2026). Sensitive Visual Detection of Breast Cancer Cells via a Dual-Receptor (Aptamer/Antibody) Lateral Flow Biosensor. *Biosensors (Basel)*, 16(2).

<https://doi.org/10.3390/bios16020085>