

Strategic Design, Formulation and Optimization of Anastrozole Solid Lipid Nanoparticles for Breast Cancer Therapy

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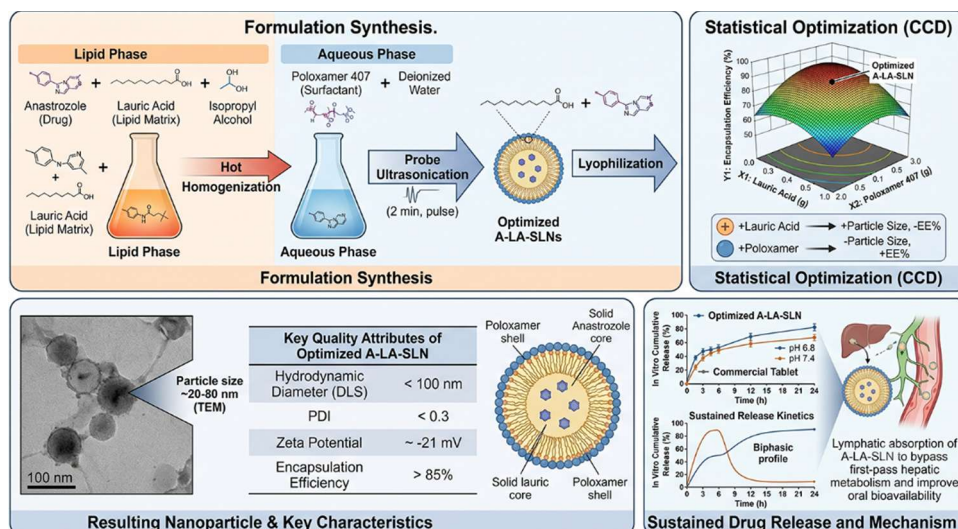
Abstract

Background: Anastrozole is a gold-standard postoperative endocrine therapy for hormone receptor-positive breast cancer in postmenopausal individuals. However, its therapeutic utility is severely restricted, necessitating frequent high-dose systemic administration which triggers severe musculoskeletal and gastrointestinal toxicities. **Objectives:** The goal of this study was to create, statistically optimize, physicochemically characterize Anastrozole-loaded solid lipid nanoparticles (A-LA-SLNs) utilizing lauric acid matrix that can exploit lymphotropic absorption pathways. **Materials and Methods:** A-LA-SLNs were fabricated using a hot homogenization-ultrasonication technique. To evaluate the influence of the independent variables, we employed a two-factor, three-level Central Composite Design (CCD). Independent variables were lipid concentration and surfactant concentration. Entrapment efficiency, hydrodynamic particle size, and zeta potential were the critical quality attributes. Physicochemical characterizations were performed and in vitro release was monitored using dialysis bag diffusion across multiple physiological pH conditions. **Results:** The optimized A-LA-SLN formulation demonstrated a particle size of 96.90 nm, a robust negative zeta potential of -21.0 mV, and an elevated entrapment efficiency exceeding 85%. FTIR and Raman spectral profiles confirmed structural compatibility and uniform molecular dispersion of Anastrozole within the fatty acid core. In vitro dissolution profiles in simulated intestinal fluids (pH 6.8 and 7.4) demonstrated highly controlled, sustained release kinetics over 24 h compared to immediate, uncontrolled dissolution in conventional commercial tablets. **Conclusion:** The developed lauric acid-based nanocarrier effectively bypassed the physicochemical limitations of conventional Anastrozole. By offering sustained-release kinetics and leveraging the inherent lymphatic affinity of medium-chain fatty acids, this delivery platform holds strong potential to enhance oral bioavailability and mitigate systemic off-target toxicities in clinical breast cancer management.

Keywords: Anastrozole, breast cancer, central composite design, lauric acid, lymphatic targeting, solid lipid nanoparticles.

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Graphical abstract



1. Introduction

Cancer remains one of the primary drivers of global mortality. According to data from the World Health Organization (WHO), malignant neoplasms accounted for an estimated 1.9 million deaths worldwide in 2021¹. Epidemiological trends indicate distinct sex-specific patterns in oncology; female patients present the highest susceptibility to breast, lung, and thyroid malignancies, whereas male patients are predominantly afflicted by lung, colorectal, liver, and prostate cancers. Clinically, early-stage diagnosis remains the single most critical determinant of therapeutic success, as intervention during localized stages significantly enhances treatment efficacy and long-term survival. Current clinical modalities for cancer management primarily rely on a combination of surgical resection, chemotherapy, radiation therapy, and endocrine modulation².

For hormone receptor-positive breast cancer, particularly in postmenopausal demographics, the potent nonsteroidal aromatase inhibitor (AI) Anastrozole, chemically designated as 2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropanenitrile), is widely prescribed as a gold-standard adjuvant therapy³. Anastrozole functions via it competitively inhibit the cytochrome P450 aromatase enzyme, thereby suppressing the peripheral biosynthesis of estrogen from androgen substrates⁴. This systemic suppression of estrogen biosynthesis deprives estrogen-dependent tumor cells of their primary growth stimulus. Because peripheral tissues, rather than functional ovaries are the principal sites of estrogen synthesis in postmenopausal women, aromatase inhibition is highly effective in this patient cohort⁵.

Despite its clinical efficacy, the therapeutic utility of Anastrozole is severely bottlenecked by its physicochemical profile. Under the Biopharmaceutical Classification System (BCS), Anastrozole is categorized as a Class III drug, characterized by high aqueous solubility but poor membrane permeability. This low permeability restricts its oral bioavailability and necessitates frequent, high-dose systemic administration. Consequently, patients often experience severe, dose-dependent adverse events, including gastrointestinal distress (nausea, emesis, and abdominal pain), vasomotor symptoms (hot flushes), and musculoskeletal complications (arthralgia, joint stiffness). More critically, prolonged estrogen deprivation accelerates bone mineral density (BMD) depletion, predisposing patients to severe osteoporosis and skeletal fractures. These therapeutic limitations underscore an urgent demand for advanced drug delivery strategies capable of enhancing the therapeutic index of Anastrozole while minimizing off-target toxicities.

To address these challenges, nanotechnology-driven drug delivery systems have emerged as promising vectors in oncology, offering enhanced pharmacokinetics, passive tumor targeting via the enhanced permeability and retention (EPR) effect, and the capacity to circumvent multidrug resistance (MDR) pathways. Specifically, solid lipid nanoparticles (SLNs) have demonstrated notable efficacy in mitigating transporter-mediated drug efflux. This is achieved through their inherent ability to bypass apical efflux pumps, such as P-glycoprotein (P-gp), or via the co-encapsulation of active P-gp inhibitors^{2,6,7}.

Selecting an optimal lipid matrix is foundational to SLN

performance. Lauric acid (LA), a 12-carbon saturated medium-chain fatty acid (MCFA) that constitutes approximately 45–53% of coconut oil, presents unique biopharmaceutical advantages^{8,9}. Historically recognized in traditional Ayurvedic medicine for its diverse therapeutic properties, lauric acid exhibits distinct absorption kinetics. Unlike long-chain fatty acids (LCFAs) that rely entirely on lymphatic transport, or shorter MCFAs (C₈–C₁₀) that drain directly into the portal vein, lauric acid exhibits an intermediate, dual-pathway absorption profile: roughly 25–30% enters the portal circulation, while the remaining 70–75% is absorbed via the intestinal lymphatic system^{10–14}.

This pronounced affinity for the lymphatic pathway is highly advantageous for oral delivery systems. By routing the majority of the lipid matrix through the mesenteric lymph nodes, the encapsulated drug partially avoids first-pass hepatic metabolism, thereby augmenting systemic bioavailability. Furthermore, this lymphatic transport mechanism is strategically suited for targeting breast cancer malignancies, which frequently metastasize via regional lymphatic networks^{15,16}. Additionally, lauric acid exhibits high biocompatibility, as evidenced by its substantial presence in human breast milk (~6.2% of total lipid content) and its documented endogenous antimicrobial properties¹⁷.

Building upon these rationales, the objective of the present study was to develop, optimize, and evaluate Anastrozole-loaded solid lipid nanoparticles utilizing a lauric acid matrix. To systematically assess and optimize the formulation parameters, a Factorial Design was employed¹⁸. This approach aims to engineer an SLN framework capable of accelerating trans-epithelial transport and enhancing oral bioavailability, ultimately providing a superior therapeutic alternative to conventional commercial oral tablets.

Materials and Methods

Anastrozole was procured from Yarrow Chem Products (Mumbai, India). Pure lauric acid (melting point: 44–46°C) was purchased from SD Fine-Chem Limited (Mumbai, India). Pluronic® F-127 (Poloxamer 407) was obtained from Sigma-Aldrich (Bangalore, India). Isopropyl alcohol was sourced from Merck India Ltd. (Mumbai, India). All other chemicals, solvents, and reagents utilized throughout the study were of analytical grade and used as received without undergoing further purification. High-purity water utilized in all experimental procedures was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). This study did not involve human or animal subjects; therefore, no ethical approval was required.

Preparation of Solid Lipid Nanoparticle Formulations

Blank solid lipid nanoparticles (LA-SLNs) and Anastrozole-loaded solid lipid nanoparticles (A-LA-SLNs) were prepared using an established hot homogenization-ultrasonication technique^{18,19}. Briefly, the organic lipid phase was prepared by dissolving 25 mg of Anastrozole and a pre-determined quantity of lauric acid in 2 mL of isopropyl alcohol. Concurrently, the aqueous phase was prepared by dissolving a specified quantity of surfactant (Poloxamer 407) in 50 mL of deionized water under continuous stirring in a beaker.

The organic lipid phase was subsequently introduced drop-wise into the aqueous phase under constant magnetic stirring to form a primary emulsion. To achieve a stable nano-emulsion, the mixture was subjected to probe ultrasonication at an output power of 100W for 2 min in pulse mode (10 s intervals) using an ultrasonicator (Model MISONIX3000, Misonix Inc., Farmingdale, NY, USA). The resulting nano-emulsion was allowed to cool to room temperature and subsequently lyophilized using a freeze dryer (FreeZone 6, LABCONCO, Kansas City, MO, USA). The obtained dry lyophilized nanoparticles were stored for subsequent evaluation of particle size, zeta potential, entrapment efficiency, and solid-state physicochemical characterization.

Optimization of SLN Formulations via Central Composite Design (CCD)

Statistical optimization and experimental design were executed using Stat-Ease Design-Expert® software (version 11.12.2.0, Stat-Ease Inc., Minneapolis, MN, USA). A two-factor, three-level (-1, 0, +1) Central Composite Design (CCD) was employed to systematically model the formulation¹⁸. The independent variables selected were lipid concentration (Lauric acid, X₁) and surfactant concentration (Poloxamer 407, X₂). The operational levels and variable boundaries are delineated in Table 1. A similar study suggested that doxorubicin loaded albumin nanoparticles fabricated by CCD technique was a promising viable alternative for drug delivery application to use for cancer therapy²⁰.

Table 1: Central composite matrix for the solid lipid nanoparticles

Experiment	SLN code	Poloxamer content (coded)	Lauric Acid content (coded)	Poloxamer content (gm)	Lauric Acid content (gm)
1	F1	-1	-1	1	0.8
2	F2	1	-1	2	0.8
3	F3	-1	1	1	1.8
4	F4	1	1	2	1.8
5	F5	-1	0	1	1.3
6	F6	1	0	2	1.3
7	F7	0	-1	1.5	0.8
8	F8	0	1	1.5	1.8
9	F9	0	0	1.5	1.3
10	F10	0	0	1.5	1.3
11	F11	0	0	1.5	1.3
12	F12	0	0	1.5	1.3
13	F13	0	0	1.5	1.3

Per the generated design matrix, a total of 13 experimental runs were executed (Table 2). The systematic optimization workflow encompassed design generation, experimental execution, response analysis, and post-analysis verification. Analysis of variance (ANOVA) was utilized to evaluate the statistical significance of the models based on F-values and p-values (p < 0.05). The mathematical relationship between the independent factors and

dependent responses was modeled using a second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Y represents the predicted response; β_0 is the intercept; β_1 and β_2 are the linear coefficients; β_{11} and β_{22} denote the quadratic (squared) coefficients; β_{12} is the interaction coefficient; and X₁ and X₂ represent the independent variable levels.

Table 2: The effect of independent factors on the responses of the formulated solid lipid nanoparticles of anastrozole

	Factor 1	Factor 2	Response 1	Response 2	Response 3
SLN code	A: Poloxamer	B: Lauric Acid	Entrapment Efficiency	Particle Size	Zeta Potential
	gm	gm	%	nm	mV
F1	1	0.8	81.66	96.90	-21.56
F2	2	0.8	86.65	85.25	-19.78
F3	1	1.8	65.35	156.25	-20.85
F4	2	1.8	69.65	116.59	-22.36
F5	1	1.3	66.62	124.56	-19.85
F6	2	1.3	83.72	89.15	-20.56
F7	1.5	0.8	85.26	87.26	-21.33
F8	1.5	1.8	72.54	119.69	-22.51
F9	1.5	1.3	70.64	112.12	-23.25
F10	1.5	1.3	71.52	110.21	-19.95
F11	1.5	1.3	69.22	109.68	-20.85
F12	1.5	1.3	70.12	111.50	-21.65
F13	1.5	1.3	68.26	110.86	-22.65

Model fit was rigorously assessed via fit statistics, ensuring that the difference between the adjusted R^2 and predicted R^2 values was less than 0.20, and that the adequate precision value exceeded 4.0. Response surface methodology (RSM) 3D plots were analyzed to interpret the multi-variable interactions and navigate constraints. To validate the predictive capability and precision of the optimized model, confirmation experiments were performed in triplicate, and the experimental outcomes were compared against the software-predicted values. The critical quality attributes (CQAs) selected as dependent responses were encapsulation efficiency (Y_1), hydrodynamic particle size (Y_2), and zeta potential (Y_3).

Physicochemical and Morphological Characterization

The hydrodynamic particle size (z-average) and zeta potential of the fabricated LA-SLNs and A-LA-SLNs were determined by dynamic light scattering (DLS) and electrophoretic light scattering, respectively, using a Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25°C. All measurements were performed in triplicate and reported as mean values. During analysis, the dispersant (water) viscosity was defined as 0.89 cP and the refractive index of lauric acid was set to 1.423^{21,22}.

The morphological attributes and surface topography of the optimized A-LA-SLNs were evaluated via transmission electron microscopy (TEM; Hitachi H-7650, Hitachi High-Technologies Corp., Tokyo, Japan). A droplet of the diluted nano-dispersion was cast onto a carbon-coated copper grid, allowed to air-dry completely, and imaged at an accelerating voltage suitable for nano-scale resolution.

Solid-state and intermolecular interactions among the raw components and formulations were analyzed. Fourier-transform infrared (FTIR) spectra of pure lauric acid, Poloxamer 407, pure Anastrozole, physical mixtures, blank LA-SLNs, and A-LA-SLNs were recorded using an FTIR spectrophotometer (Nicolet 5700, Thermo Fisher Scientific, Waltham, MA, USA). Complementary structural evaluations were conducted using a confocal Raman microscope (Alpha 300RA, WITec GmbH, Ulm, Germany).

A differential scanning calorimeter (DSC, Hitachi, Japan) was used to analyze the thermal properties wherein the sample was heated under nitrogen (60mL/min) at a scanning rate of 10°C/min from -4°C to 150°C. Using thermogram data, the crystallinity index (%) of optimized A-LA-SLNs was calculated.

Determination of Entrapment Efficiency and Drug Loading

The entrapment efficiency (EE %) of Anastrozole within the lipid matrix was determined indirectly by quantifying the untrapped (free) drug present in the aqueous supernatant. The aqueous nano-dispersion was transferred

into an Amicon® Ultra-4 centrifugal filter unit (Millipore, Bedford, MA, USA) equipped with a 30 kDa molecular weight cut-off (MWCO) cellulose membrane. The units were centrifuged, and the collected filtrate containing the untrapped free drug was analyzed using a UV-Visible spectrophotometer. The concentration of free Anastrozole was calculated utilizing a validated calibration curve (linear range: 0.05 to 3.75 µg/mL; $R^2 = 0.9989$). The percentage entrapment efficiency was calculated as follows:

$$EE \% = \frac{\text{Total Drug Added} - \text{Free Untrapped Drug}}{\text{Total Drug Added}} \times 100$$

For the determination of drug loading capacity, lyophilized formulations prepared with varying initial concentrations of active drug (1 mg, 5 mg, and 10 mg) were lysed in an appropriate solvent system to liberate the entrapped therapeutic payload. The absolute drug content was determined spectrophotometrically via a secondary calibration curve (linear range: 0.0018 to 3.75 µg/mL; $R^2 = 0.9943$).

In Vitro Drug Release Dynamics

The *in vitro* release profile of Anastrozole from the optimized A-LA-SLNs was investigated using the equilibrium dialysis bag diffusion method. Briefly, an amount of lyophilized A-LA-SLN equivalent to 20 mg of formulation was homogeneously re-dispersed in 2 mL of phosphate-buffered saline (PBS, pH 7.4). The dispersion was sealed within a cellulose dialysis bag with an MWCO of 14,000 Da (Sigma-Aldrich, India). The sealed bag was subsequently immersed in a receptor compartment containing 10 mL of PBS (pH 7.4) maintained at a physiological temperature of $37 \pm 0.5^\circ\text{C}$ under continuous magnetic stirring.

At predetermined temporal intervals, aliquots of 200 µL were sampled from the receptor medium. To maintain sink conditions and a constant receptor volume, an equivalent volume (200 µL) of fresh, pre-warmed PBS (pH 7.4) was immediately replenished after each sampling event. The concentration of Anastrozole released into the sampling aliquots was quantified via UV-Visible spectroscopy, and the cumulative percentage of drug release was plotted as a function of time. Finally, the drug release kinetics were analyzed, and the corresponding release rate constants were determined.

Results

Characterization of LA-SLN and A-LA-SLN

The average size of drug loaded nanoparticles was 96.90 nm with a distribution from around 10 nm to 300 nm (figure 1a). This was correlating with the TEM image which was showing a particle size of around 20nm to 80nm (figure 1b). The zeta potential of the LA-SLN suspension in water was found to be -21.56 mV.

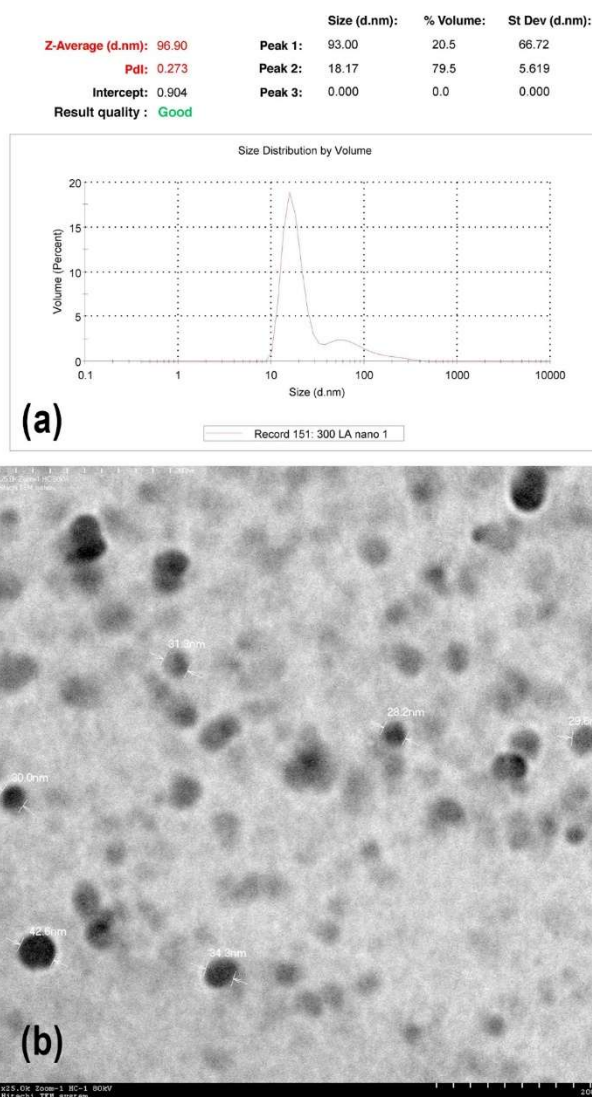


Figure 1: (a) Size distribution of nanoparticles by dynamic light scattering, (b) Transmission electron micrograph of nanoparticles

The FTIR spectra and Raman spectra of the drug loaded nanoparticles were compared with the spectra of individual components. FTIR spectra of anastrozole (figure 2a) exhibited the characteristic absorption bands (major peaks) at wave numbers, 3099.7 cm^{-1} due to aromatic C–H stretch, 2982.05 and 2918.4 cm^{-1} due to CH_3 aliphatic stretch, 2234.64 cm^{-1} (medium C:N stretch), 1604.83 cm^{-1} (skeletal vibrations) due to C=C of aromatic ring, 1572.04 and 1502.6 cm^{-1} due to aromatic ring, 1537.32 cm^{-1} due to C=N stretching, 1465.95, 1357.93 and 1390.72 cm^{-1} due to gem dimethyl groups, 1273.06 and 1201.69 cm^{-1} due to tertiary butyl type skeletal vibration, and 761.91 cm^{-1} due to out of plane C–H bend (aromatic ring), respectively. The FTIR spectra of lauric acid (figure 2b) has the symmetrical stretching vibration of the –CH group at 2851 cm^{-1} . The absorption peaks at 1291 and 1700 cm^{-1} of lauric acid indicate the presence of –

OH and C=O, respectively. The FTIR spectra of drug loaded nanoparticles is shown in figure 2c.

The Raman spectra of drug loaded nanoparticles is shown in figure 2d. Characteristic for all fatty acids is the presence of two intense bands between 1050 and 1150 cm^{-1} arising from $\nu(\text{C-C})$ stretch vibrations (1063, 1126), one band at 1296 cm^{-1} arising from $\gamma(\text{CH}_2)$ twisting and a group of bands between 1400 and 1500 cm^{-1} caused by $\gamma(\text{CH}_2)$ wagging, $\gamma(\text{CH}_2)$ scissoring as well as $\delta(\text{CH}_2)$ and $\delta(\text{CH}_3)$ deformation vibrations. Typically, carbonyl stretch frequencies of aliphatic compounds are found above 1700 cm^{-1} . However, the carbonyl stretch frequency $\nu(\text{C=O})$ of the lauric acid is red-shifted to 1633 cm^{-1} and shows only moderate Raman intensity indicating a local environment in which the carbonyl is hydrogen bonded.

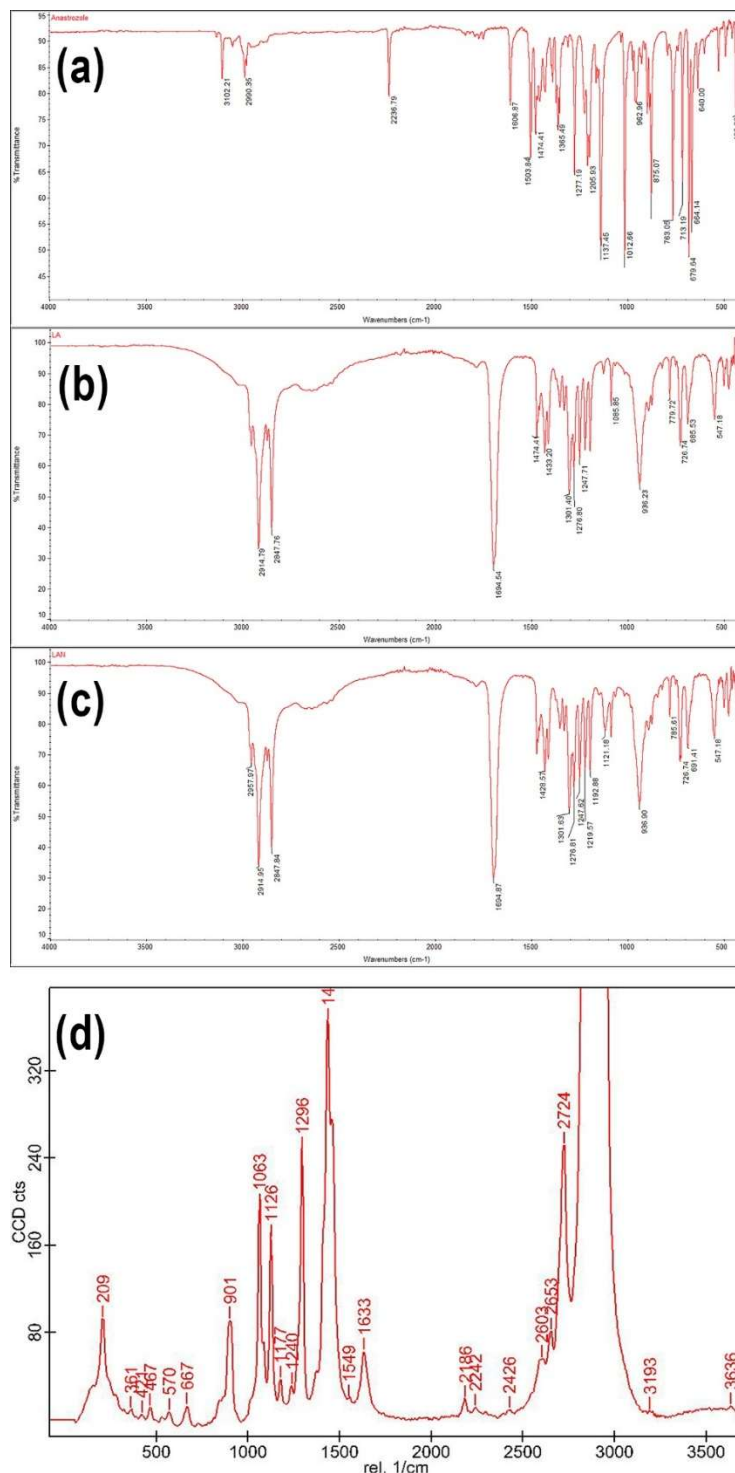


Figure 2: (a) FTIR spectra of the drug, anastrozole, (b) lauric acid and (c) drug loaded nanoparticles, (d) Raman spectra of drug loaded nanoparticles

Statistical evaluation of the experimental design

The responses obtained from the design matrix related to entrapment efficiency, particle size and zeta potential were statistically evaluated using Design Expert® (ver 11.12.2.0) software (StatEase, MN, USA).

Response: entrapment efficiency

A quadratic model was suggested for entrapment efficiency¹⁸. The model proposed the following polynomial

equation for percentage entrapment efficiency:

$$Y1 = 75.61 + 2.40A - 6.84B - 1.42AB + 5.91A^2 - 2.86B^2$$

Equation 1

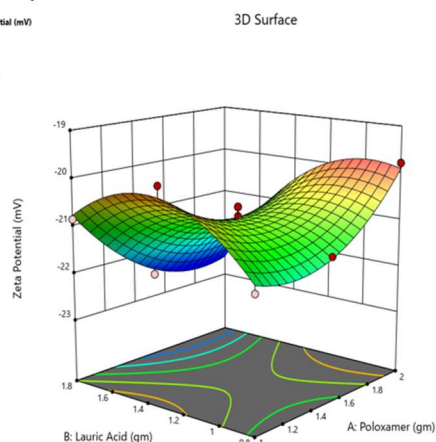
where Y1 is the percentage entrapment efficiency, A is the lauric acid content (g) and B is the content of surfactant (g).

As evidenced by the model coefficients and the response surfaces (Figure 3a), the surfactant concentration (X₁) exerted a highly pronounced positive effect on drug

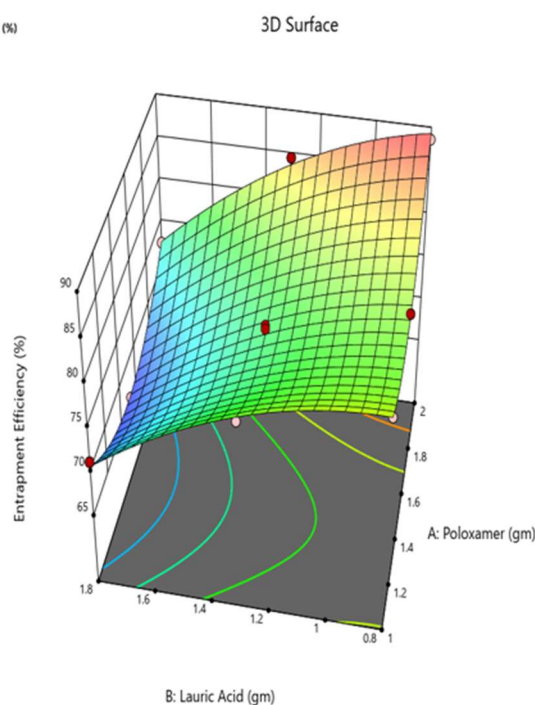
encapsulation. Increasing the Poloxamer 407 concentration from 1.0 g to 2.0 g significantly increased the entrapment efficiency, peaking at 86.65 (Formulation F2). This behavior is attributed to the surfactant's ability to stabilize the oil-

water interface, reducing leakage of the hydrophilic Class III drug into the external aqueous phase. On the other hand, the entrapment efficiency dropped significantly as the concentration of lauric acid (X2) was raised.

Factor Coding: Actual
Response: Zeta Potential (mV)
Design Points:
● Above Surface
○ Below Surface
-22.51 -19.78



Factor Coding: Actual
Response: Entrapment Efficiency (%)
Design Points:
● Above Surface
○ Below Surface
65.54 88.65



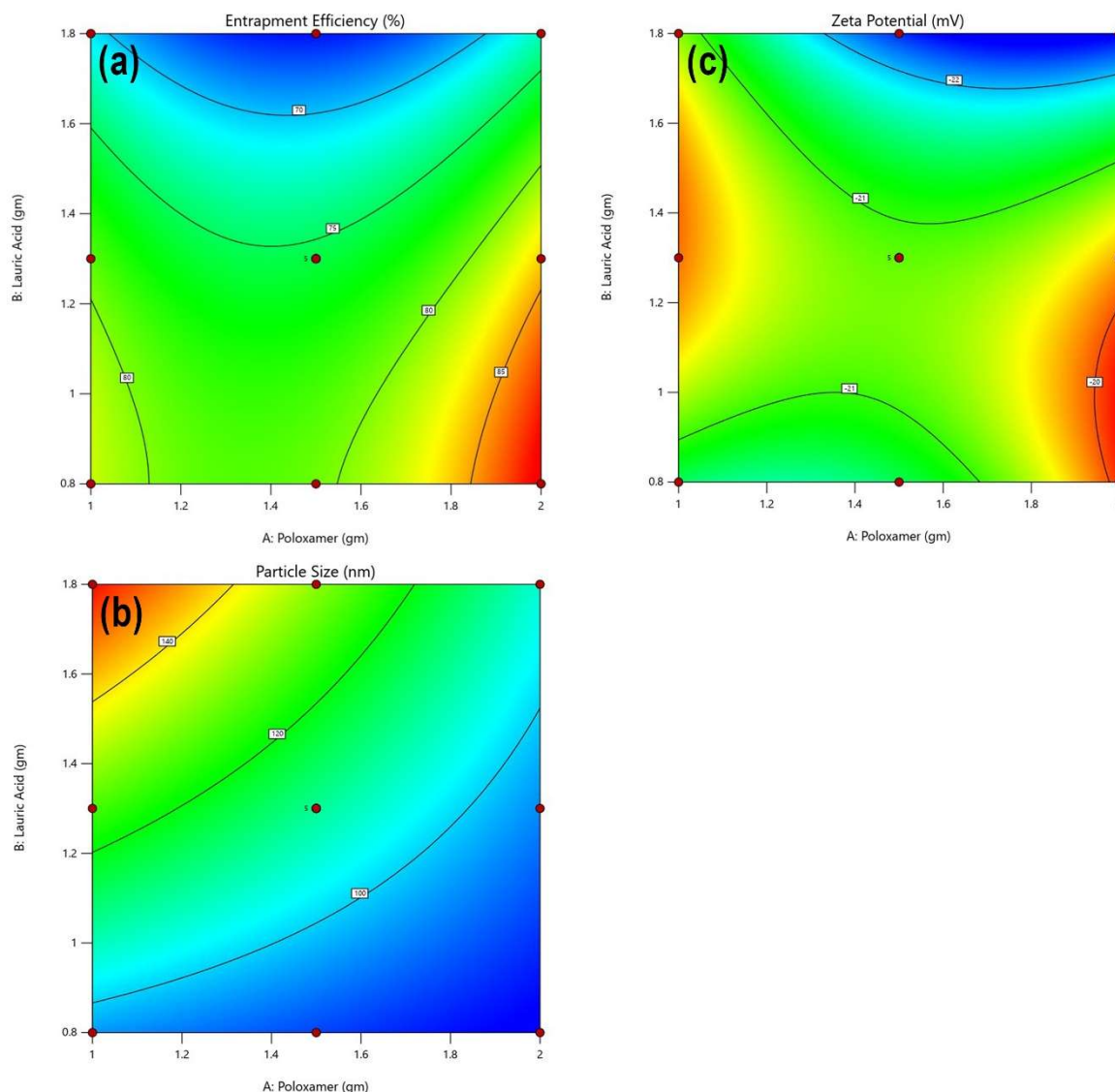


Figure 3: (a) Contour plot for the response, entrapment efficiency, (b) particle size and (c) zeta potential

Response: particle size

A two-factor interaction (2FI) model was suggested by the software for particle size¹⁸. The model proposed the following equation for particle size:

$$Y2 = 110.43 - 15.39A + 20.42B - 9.35AB$$

Equation 2

where Y2 is the particle size, A is the lauric acid content (g) and B is the content of surfactant (g).

The statistical analysis revealed that both independent variables significantly dictated the final nano-dispersion size (Figure 3b). The surfactant concentration (X_1) demonstrated an inverse relationship with particle size; higher concentrations of Poloxamer 407 drastically reduced the particle diameter (e.g., minimizing to 85.25 nm in F2). In contrast, increasing the lauric acid concentration (X_2) directly increased the particle size (Y_2).

Response: zeta potential

A quadratic model was suggested by the software

for drug loading¹⁸. The model proposed the following equation for drug loading:

$$Y3 = -20.89 - 0.73A - 0.51B - 0.82AB + 0.72A^2 - 0.99B^2$$

Equation 3

where Y3 is the zeta potential, A is the lauric acid content (g) and B is the content of surfactant (g). The zeta potential values across all 13 experimental runs remained consistently negative, tightly bound within a narrow corridor ranging from -19.78 mV to -23.25 mV (Table 2, Figure 3c). Statistical evaluation confirmed that neither Poloxamer 407 concentration (X_1) nor lauric acid content (X_2) exerted a statistically significant ($p > 0.05$) effect on the zeta potential.

In Vitro Release Kinetics and Comparative Dissolution

The cumulative *in vitro* release profiles of Anastrozole from the optimized A-LA-SLN formulation and conventional commercial tablets were thoroughly evaluated across three distinct physiological pH environments over a 24 h period

(Figures 4a and 4b). At an acidic gastric pH (pH 1.2), both formulations exhibited restricted drug release. At simulated physiological intestinal pH conditions (pH 6.8 and pH 7.4), the A-LA-SLN formulation demonstrated a highly controlled, and sustained drug release profile. An initial mild burst

release within the first 2 h was followed by a prolonged, steady Korsmeyer Peppas type release kinetics extending up to 24 h. In sharp contrast, the commercial tablet formulation exhibited rapid, uncontrolled dissolution, achieving complete drug exhaustion within the initial phases of testing.

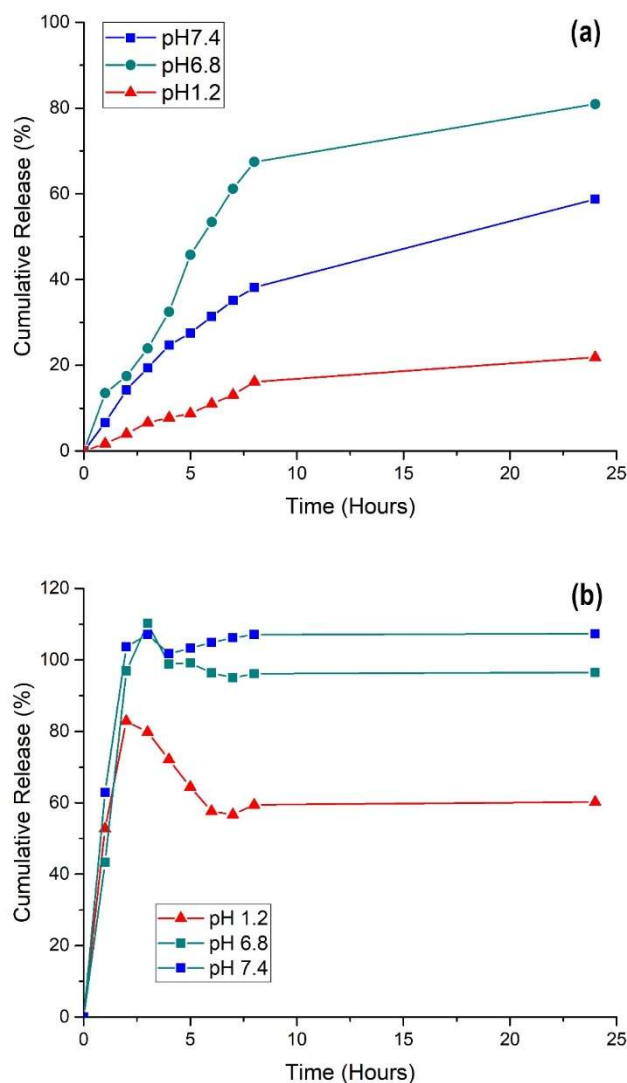


Figure 4: (a) Cumulative release of anastrozole from solid lipid nanoparticles and (b) from the tablet formulation.

Discussion

Characterization of LA-SLN and A-LA-SLN

The hydrodynamic size by dynamic light scattering (DLS) is a non-invasive, well-established technique for measuring the size and size distribution of particles in suspension. The average size of drug loaded nanoparticles was 96.90 nm with a distribution from around 10 nm to 300 nm. This was correlating with the TEM image which was showing a particle size of around 20nm to 80nm. Particle size is a key factor affecting drug release and therapeutic efficiency. Smaller particle size leads to greater drug absorption and improved therapeutic effect^{9,23}.

Oral drug delivery carriers must withstand the varying pH conditions of the gastrointestinal (GI) tract; therefore, evaluating their stability under different GI pH environments is essential⁹. In this study, the stability of LA-SLN and A-LA-SLN was investigated at different GI tract pH conditions, namely pH 1.2 (stomach), pH 6.8 (small intestine), and pH 7.4 (colon), using dynamic light scattering (DLS). DLS is widely employed not only for measuring the particle size of nanoparticles in suspension but also for assessing their stability under different pH conditions²⁴.

The particle charge in a given environment is represented by the zeta potential, which is an important

parameter indicating the stability of the LA-SLN suspension. The zeta potential of the LA-SLN suspension in water was found to be -21.56 mV. The electrostatic repulsion between the negatively charged SLNs enhances stability and prevents the aggregation of nanoparticles. The zeta potential of nanoparticles suspended in phosphate buffer (pH 7.4) was evaluated to simulate the surface charge behavior of SLNs under biological conditions⁹. Zeta potential is mainly influenced by factors such as pH, ionic strength, and the composition of the solution^{25,26}.

From the FTIR spectra, all the characteristic peaks of the drug and lipid appear in the spectra of solid dispersion of anastrozole indicating no interaction between the drug and the carrier. The Raman spectra of drug loaded nanoparticles exhibited all the characteristic peaks of fatty acids. However, the carbonyl stretch frequency $\nu(\text{C}=\text{O})$ of the lauric acid is red-shifted to 1633 cm^{-1} and shows only moderate Raman intensity indicating a local environment in which the carbonyl is hydrogen bonded. A similar characteristic peak pattern has also been reported in literature for FTIR and Raman analysis^{27,28}.

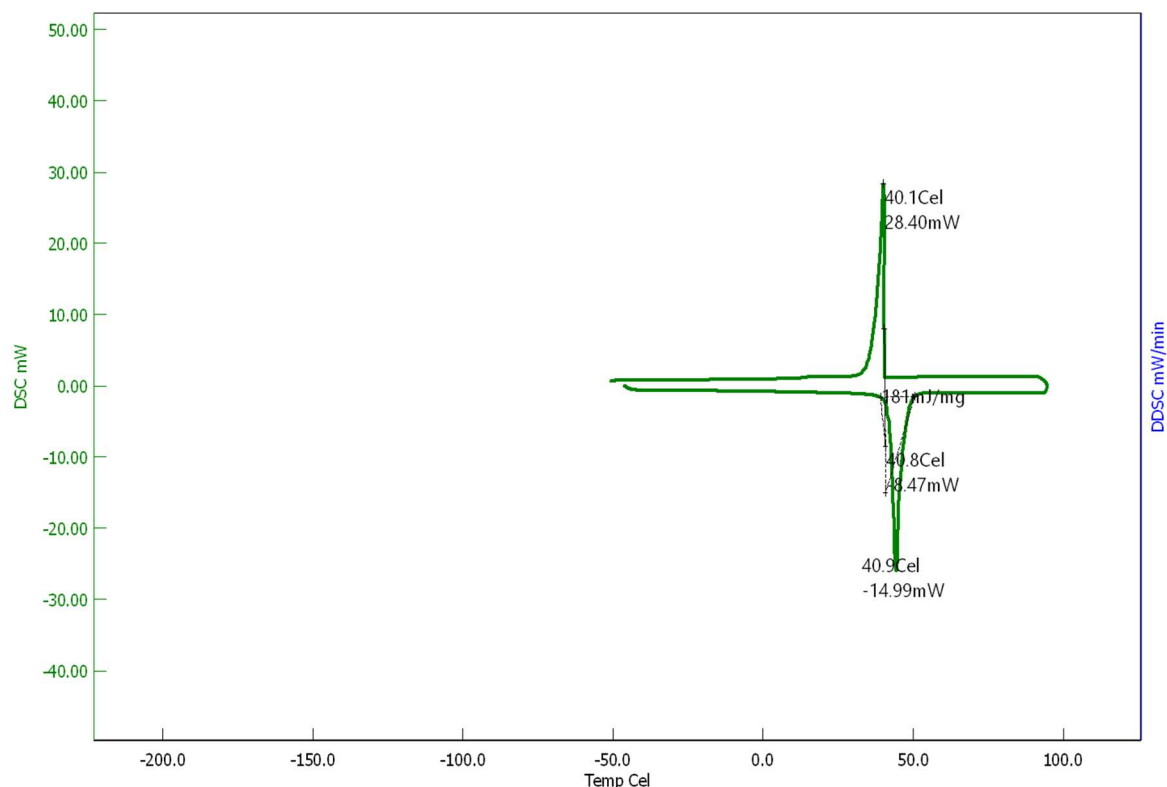


Figure 5: DSC thermogram of drug loaded lipid nanoparticles

Differential scanning calorimetry (DSC) was performed to evaluate the thermal behavior of anastrozole-loaded solid lipid nanoparticles (A-LA-SLN) prepared using lauric acid and Poloxamer 407 (figure 5). The DSC thermogram exhibited a prominent thermal transition at approximately 40.06°C , corresponding mainly to the melting of the lauric acid-based lipid matrix. The observed thermal behavior indicates that incorporation of anastrozole and Poloxamer 407 altered the molecular organization and crystalline structure of the lipid matrix. The enthalpy associated with the transition was approximately 180.77 J/g . The alteration in the thermal transition compared with the individual lipid component may be attributed to drug incorporation and interaction with the lipid matrix, resulting in modification of its crystallinity. These findings support the successful formation of the lipid-based nanoparticulate system and provide evidence of changes in the physical state of the lipid matrix following formulation.

Statistical evaluation of the experimental design

The responses obtained from the design matrix related to entrapment efficiency, particle size and zeta potential were statistically evaluated using Design Expert® (ver 11.12.2.0) software (StatEase, MN, USA)²⁹.

As evidenced, the surfactant concentration exerted a highly pronounced positive effect on drug encapsulation. Increasing the Poloxamer 407 concentration significantly increased the entrapment efficiency, peaking at 86.65 (Formulation F2). This behavior is attributed to the surfactant's ability to stabilize the oil-water interface, reducing leakage of the hydrophilic Class III drug into the external aqueous phase. On the other hand, the entrapment efficiency dropped significantly as the concentration of lauric acid was raised. This trend can be attributed to the tendency of dense lipid masses to organize into highly ordered, crystalline structures. Because a perfect crystal lattice lacks the structural defects and interstitial gaps needed to hold the guest molecules, the Anastrozole is effectively excluded from the lipid matrix.

The surfactant concentration demonstrated an inverse relationship with particle size; higher concentrations of Poloxamer 407 drastically reduced the particle diameter (e.g., minimizing to 85.25 nm in F2). This is due to the enhanced reduction in interfacial tension during homogenization, which prevents the coalescence of newly formed lipid droplets. In contrast, increasing the lauric acid concentration directly increased the particle size. The increased viscosity of the internal organic phase at higher lipid concentrations limits the mechanical shear efficiency of the probe ultrasonicator, yielding larger lipid droplets (e.g., 156.25 nm in F3).

The zeta potential values across all 13 experimental runs remained consistently negative, tightly bound within a narrow corridor ranging from -19.78 mV to -23.25 mV. Statistical evaluation confirmed that neither Poloxamer 407 concentration nor lauric acid content exerted a statistically significant ($p > 0.05$) effect on the zeta potential. The net negative charge is fundamentally dictated by the terminal carboxylic groups of lauric acid exposed at the nanoparticle boundary. The uniform magnitude of approximately -21 mV indicates strong electrostatic repulsion among the particles, which provides robust physical stability against agglomeration and flocculation.

In Vitro Release Kinetics and Comparative Dissolution

The cumulative *in vitro* release profiles of Anastrozole from the optimized A-LA-SLN formulation and conventional commercial tablets were thoroughly evaluated across three distinct physiological pH environments over a 24 h period. At an acidic gastric pH (pH 1.2), both formulations exhibited restricted drug release. For the A-LA-SLNs, this suppressed release is primarily dictated by the low solubility of Anastrozole in highly acidic microenvironments combined with the physical barrier properties of the solid lauric acid core, which structurally impedes the penetration of the acidic dissolution medium into the nanoparticle matrix. At simulated physiological intestinal pH conditions (pH 6.8 and pH 7.4), the A-LA-SLN formulation demonstrated a highly controlled, biphasic, and sustained drug release profile. An initial mild burst release within the first 2 h was followed by a prolonged, steady Korsmeyer Peppas type release kinetics extending up to 24 h. The optimized formulation of A-LA-SLN showed 35.52% drug release in pH 1.2 within 24h, 92.51% drug release within 24 h in pH 6.8, and 73.52% drug release within 24 h in pH 7.4. At pH 6.8 phosphate buffer the optimized formulation (F2) shows zero order kinetics the R^2 value 0.7898, first order kinetics R^2 value 0.9257, korsmeyer peppas R^2 value 0.9427 and Higuchi R^2 value 0.9247. Based on the R^2 value Korsmeyer Peppas's was the best fit model for formulation as shown in figure 6.

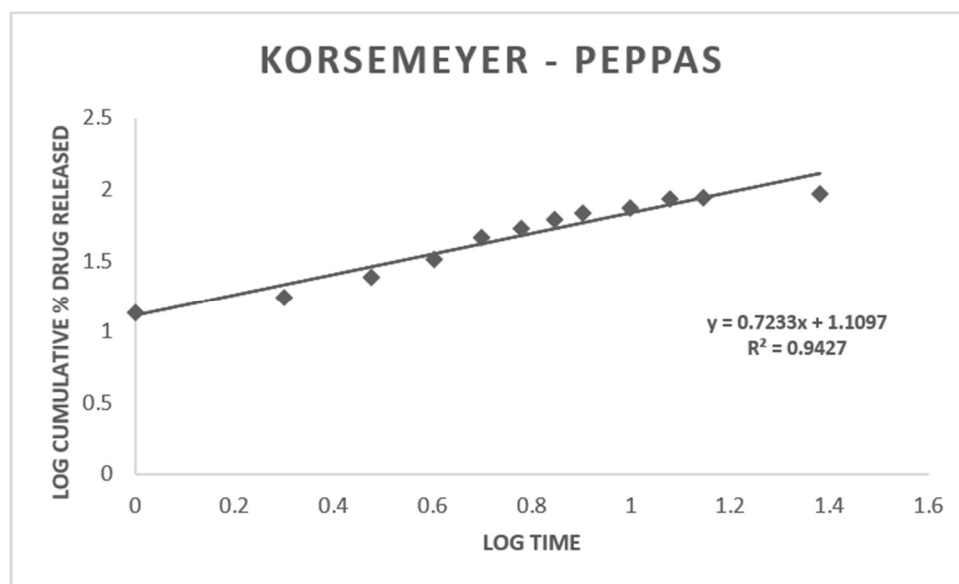


Figure 6: Based on the R^2 value Korsmeyer Peppas's was the best fit model for the drug release profile of the formulation

In sharp contrast, the commercial tablet formulation exhibited rapid, uncontrolled dissolution, achieving complete drug exhaustion within the initial phases of testing. The sustained release kinetics provided by the lauric acid matrix are ideal for reducing the localized gastrointestinal irritation and fluctuating plasma concentration spikes typically associated with conventional oral Anastrozole therapies.

Conclusion

In this study, Anastrozole-loaded solid lipid nanoparticles (A-LA-SLNs) utilizing a biocompatible lauric acid matrix were successfully fabricated and

systematically optimized via a Central Composite Design³⁰. The engineered nanocarriers satisfied all critical quality attributes, displaying an optimal sub-micron hydrodynamic diameter (< 100 nm), robust physical stability (zeta ~ -21 mV), and an elevated entrapment efficiency ($> 85\%$) aligning with standard lipid-based nanocarrier standards for aromatase inhibitors³¹. Solid-state spectroscopic evaluations confirmed the structural integrity and chemical compatibility of the drug within the lipid core.

The primary clinical limitations of conventional oral Anastrozole, specifically its poor membrane

permeability (BCS Class III), rapid systemic spikes, and associated dose-dependent gastrointestinal and musculoskeletal toxicities, could be effectively mitigated by this nano-formulation. By offering a highly regulated, sustained release profile, these lauric acid-based solid lipid nanoparticles present a promising, biocompatible delivery platform capable of improving therapeutic outcomes and patient compliance in endocrine-based breast cancer management³². A similar study of anastrozole loaded PLGA nanoparticles exhibited remarkable decrease in cell viability and the pharmacokinetics study revealed the long-time circulation of anastrozole loaded polymeric nanoparticles³³.

The development of surfactant-modified solid lipid nanoparticles leveraging medium-chain fatty acid matrices represents a vital frontier in overcoming traditional oral delivery barriers³⁴. Future research will focus on translating these optimized A-LA-SLN formulations into comprehensive *in vivo* pharmacokinetic and biodistribution studies to definitively quantify their absorption pathways. Given that lauric acid (C12) inherently possesses a unique dual-absorption profile, surface modification of these SLNs can be further tailored to maximize uptake via the intestinal lymphatic system. By routing the therapeutic payload directly through the mesenteric lymph nodes into the systemic circulation, first-pass hepatic metabolism can be bypassed, drastically enhancing oral bioavailability³⁵. More critically, because breast cancer cells aggressively exploit regional lymphatic networks for early metastasis, utilizing a lymphotropic lauric acid nanocarrier opens up an innovative therapeutic avenue. This strategy allows for the direct, site-specific delivery of aromatase inhibitors to secondary lymphatic tumor deposits, potentially inhibiting regional recurrence while significantly reducing the systemic dosage required for long-term safety and efficacy.

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Abbreviations:

- **A-LA-SLNs:** Anastrozole-loaded solid lipid nanoparticles
- **ANOVA:** Analysis of Variance
- **BCS:** Biopharmaceutical Classification System
- **CCD:** Central Composite Design
- **FTIR:** Fourier-transform infrared
- **HR+:** Hormone receptor-positive
- **LA:** Lauric acid
- **MCFA:** Medium-chain fatty acid
- **SLN:** Solid lipid nanoparticles

Conflict of interest:

The authors declare no conflicts of interest regarding the publication of this paper.

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Author Contributions:

SSK designed the study and performed the experiments. AKM and SSD supervised the research. All authors reviewed and approved the final manuscript.

Summary:

This study successfully developed Anastrozole-loaded lauric acid solid lipid nanoparticles using a hot homogenization-ultrasonication technique, optimized via Central Composite Design. The resulting nanoparticles (<100 nm, robust negative surface charge, and >85% encapsulation efficiency) exhibited controlled, biphasic, and sustained release over 24 hours, presenting a highly promising alternative to conventional Anastrozole therapies for breast cancer management.

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