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RESEARCH ARTICLE

Design, Synthesis, and Evaluation of Letrozole-Loaded Nanoemulsion for Targeted Breast Cancer Therapy

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Abstract: Breast cancer remains one of the most prevalent malignancies among women, with hormone receptor–positive subtypes constituting the majority of cases. Letrozole, a potent non-steroidal aromatase inhibitor, effectively suppresses estrogen biosynthesis but suffers from poor aqueous solubility and low oral bioavailability, limiting its clinical efficacy. The present study aimed to design, synthesize, and evaluate a Letrozole-loaded Nanoemulsion for enhanced solubility, stability, and targeted delivery in breast cancer therapy. A Box–Behnken Design (BBD) was employed to optimize three formulation variables oil (Capryol 90), surfactant (Tween 80), and co-surfactant (Transcutol P) influencing particle size, encapsulation efficiency, and drug release. Fifteen experimental runs were generated using Design-Expert® software and scanning electron microscopy (SEM). The optimized formulation (F16) exhibited a particle size of 180.75 ± 1.5 nm, encapsulation efficiency of 91.85 ± 0.4%, and cumulative drug release of 76.25 ± 0.6% over 12 hours, demonstrating close agreement between predicted and experimental values (<3% error). SEM revealed uniform, spherical droplets with smooth morphology, confirming homogeneity and physical stability. Statistical analysis (ANOVA) indicated significant effects of oil and surfactant concentration on particle size and entrapment efficiency ($p < 0.05$). The optimized Nanoemulsion provided improved solubility, sustained release, and potential for enhanced bioavailability of Letrozole, supporting its application as a targeted nanocarrier system for breast cancer therapy.

Keywords: Letrozole, Nanoemulsion, Box–Behnken Design, Targeted Drug Delivery, Breast Cancer, Optimization, Encapsulation Efficiency

INTRODUCTION

Breast cancer remains a leading cause of cancer-related mortality among women worldwide, with hormone-dependent subtypes constituting a major proportion of cases [1]. Letrozole (Figure 1), a potent non-steroidal aromatase inhibitor, effectively suppresses estrogen biosynthesis and is widely used for the treatment of hormone receptor–positive breast cancer. However, its clinical efficacy is limited by poor aqueous solubility, low oral bioavailability, and variable absorption, leading to inconsistent therapeutic outcomes [2-6]. Nanotechnology-based delivery systems offer a promising strategy to overcome such challenges associated with poorly soluble anticancer drugs. Among them, nanoemulsions thermodynamically stable systems composed of oil, surfactant, co-surfactant, and water are recognized for enhancing drug solubility, absorption, and targeted delivery. Their small droplet size and high surface area enable improved dissolution and cellular uptake, while minimizing systemic toxicity. In this study, a Letrozole-loaded nanoemulsion was designed, synthesized, and evaluated to enhance solubility, stability, and targeted anticancer efficacy [7]. A Box–Behnken statistical design was employed to optimize formulation parameters and assess their influence on particle size, polydispersity index, and encapsulation efficiency. The optimized formulation was characterized for physicochemical properties, in vitro drug release, and cytotoxicity, aiming to establish an effective nanoemulsion system for targeted breast cancer therapy [8].

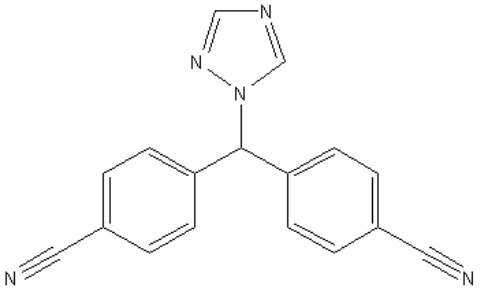

Figure 1. Chemical structure of Letrozole

MATERIALS AND METHODS

Materials

Letrozole was obtained as a gift sample from Sun Pharmaceutical Industries Ltd. (Vadodara, India). Capryol 90, Labrasol, and Transcutol P were procured from Gattefossé India Pvt. Ltd. (Mumbai, India). Tween 80 and Span 80 were purchased from Merck Life Sciences Pvt. Ltd. (Mumbai, India). All other reagents and chemicals used were of analytical grade and utilized without further purification. Double-distilled water was used throughout the study.

Preformulation Study

Preformulation studies were conducted to evaluate the physicochemical characteristics of Letrozole prior to formulation. Solubility studies were performed in water, ethanol, DMSO, and selected oil phases to determine suitable solvents for nanoemulsion preparation [9]. The melting point of Letrozole was determined using a digital melting point apparatus to confirm thermal stability under formulation conditions. The partition coefficient (Log P) of Letrozole was determined using the n-octanol/water system to assess lipophilicity, guiding the selection of the oil and surfactant system. Additionally, pH stability studies were conducted across the range of 3–8 to evaluate drug stability under physiological conditions. Overall, these preformulation studies confirmed that Letrozole is physicochemically stable, compatible with the selected excipients, and suitable for nanoemulsion encapsulation [10].

Preparation of Letrozole-Loaded Nanoemulsion

Letrozole-loaded nanoemulsions were prepared using the spontaneous emulsification method. For all batches, 150 mg of Letrozole was accurately weighed and dissolved in 10 mL of the oil phase (Capryol 90) with gentle heating at 40–45 °C to ensure complete solubilization of the drug. The surfactant (Tween 80) and co-surfactant (Transcutol P) were incorporated into the oil–drug mixture in batch-specific concentrations as defined by the experimental design (Table 1A and 1B). The aqueous phase was added dropwise to the organic phase under continuous magnetic stirring at 1500 rpm for 2–3 hours, resulting in spontaneous formation of a fine nanoemulsion. The obtained emulsion was further sonicated using a probe sonicator for three cycles of 1 minute each with 30-second intervals to reduce droplet size and improve homogeneity. The final volume of 10 mL was maintained constant across all batches. The prepared Letrozole-loaded nanoemulsions were stored in amber-coloured glass vials at room temperature until further characterization [11-13].

Experimental Design and Optimization

The formulation and optimization of Letrozole-loaded nanoemulsion were carried out using a Box–Behnken Design (BBD), a response surface methodology used to evaluate the influence of formulation variables on key responses [14]. Three independent variables were selected: oil concentration (X_1), surfactant concentration (X_2), and co-surfactant concentration (X_3). These factors were optimized to assess their effects on three dependent responses: encapsulation efficiency (Y1), particle size (Y2), and cumulative drug release at 12 hours (Y3) [15]. A total of 15 experimental runs were generated using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, USA). The design matrix and coded levels of variables are shown in **Table 1(A)** and the composition of formulations in **Table 1(B)**.

Table 1 (A). Experimental Factors and Their Levels Used in the Box–Behnken Design

Factor	Low Level (−1)	High Level (+1)	Unit
A: Oil Concentration (Capryol 90)	2.0	4.0	% v/v
B: Surfactant Concentration (Tween 80)	4.0	6.0	% v/v
C: Co-surfactant Concentration (Transcutol P)	2.0	3.0	% v/v

Table 1(B). Composition of Letrozole-Loaded Nanoemulsion Batches (F1–F15)

Batch No.	Oil (Capryol 90, % v/v)	Surfactant (Tween 80, % v/v)	Co-surfactant (Transcutol P, % v/v)
F-1	2.0	4.0	2.0
F-2	4.0	4.0	2.0
F-3	2.0	6.0	3.0
F-4	3.0	5.0	2.0
F-5	4.0	6.0	3.0
F-6	2.0	5.0	3.0
F-7	3.0	6.0	2.0
F-8	3.0	4.0	3.0
F-9	4.0	5.0	2.0
F-10	2.0	4.0	3.0
F-11	4.0	6.0	2.0
F-12	3.0	4.0	2.0
F-13	4.0	4.0	3.0
F-14	2.0	6.0	2.0

F-15	3.0	5.0	3.0

2.5 Characterization of Nanoemulsion

Particle Size and Zeta Potential

The average droplet size, polydispersity index (PDI), and zeta potential of the Letrozole-loaded nanoemulsion were determined using a dynamic light scattering (DLS) analyzer (Malvern Zetasizer Nano ZS90, UK). Samples were suitably diluted with deionized water before analysis to prevent multiple scattering effects. Measurements were performed at 25 °C in triplicate [16].

Encapsulation Efficiency (EE%)

Encapsulation efficiency was determined by ultracentrifugation of nanoemulsion samples at 15,000 rpm for 30 minutes. The supernatant was separated and analyzed for unentrapped Letrozole using a UV-Visible spectrophotometer at 240 nm [17].

In Vitro Drug Release Study

The in vitro release of Letrozole from the nanoemulsion was studied using the dialysis bag diffusion method. A fixed volume of formulation equivalent to the therapeutic dose of Letrozole was placed in a pre-soaked dialysis membrane and suspended in 100 mL of release medium (0.1 N HCl pH 1.2 for 2 hours followed by phosphate buffer pH 6.8 for 10 hours). The medium was maintained at 37 ± 0.5 °C under magnetic stirring (100 rpm). Samples (3 mL) were withdrawn at predetermined intervals and replaced with an equal volume of fresh medium. The drug content was analyzed using UV spectrophotometry at 240 nm [18].

Influence of Formulation Variables

The effects of oil, surfactant, and co-surfactant concentrations on particle size, encapsulation efficiency, and drug release were analyzed using response surface methodology (RSM). Three-dimensional (3D) surface and contour plots were generated to illustrate the interaction between independent variables and their influence on dependent responses [19].

Statistical Analysis

All experiments were carried out in triplicate, and data were expressed as mean ± standard deviation (SD). The statistical significance of the model terms was evaluated using Analysis of Variance (ANOVA), with $p < 0.05$ considered significant. Regression analysis, response plots, and desirability function analysis were generated using Design-Expert® software (Version 13) to confirm the robustness and validity of the optimized Letrozole nanoemulsion formulation.

Morphology Study

The surface morphology of the optimized Letrozole-loaded nanoemulsion (Batch F16) was analyzed using **Scanning Electron Microscopy (SEM)** (Supra 55, Carl Zeiss, Germany). A drop of the nanoemulsion was carefully placed onto a clean glass stub coated with double-sided carbon tape and allowed to air dry under vacuum. The dried sample was then sputter-coated with a thin layer of gold using a sputter coater (Quorum SC7620, UK) to enhance surface conductivity. The coated sample was observed under FE-SEM at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Preformulation Study

Solubility Studies

Solubility analysis showed that Letrozole was practically insoluble in water, moderately soluble in ethanol, and highly soluble in DMSO and the selected oil phase. Table 2 confirmed that the drug could be efficiently incorporated into the oil phase (Capryol 90) for nanoemulsion formulation.

Melting Point

The melting point of Letrozole was found to be **183–185 °C**, consistent with reported literature values, indicating thermal stability under formulation and processing conditions (Table 2).

Partition Coefficient (Log P)

The Log P value of Letrozole was determined to be **2.6**, indicating moderate lipophilicity that supports solubilization in the oil phase and enhances interaction with surfactant–co-surfactant systems.

pH Stability

Letrozole remained stable across a pH range of **3–8**, suggesting its suitability for physiological pH conditions and stability during storage.

Table 2. Preformulation characteristics of Letrozole.

Parameter	Results
Solubility (mg/mL)	Water: 0.05; Ethanol: 3.1; DMSO: 41.2; Oil: 48.7
Melting Point (°C)	183–185
Partition Coefficient (Log P)	2.6
pH Stability	pH 3–8

Evaluation of Letrozole-Loaded Nanoemulsion

Letrozole-loaded nanoemulsions were successfully prepared by the spontaneous emulsification method. The formulation parameters, particularly concentrations of oil, surfactant, and co-surfactant, significantly influenced particle size, encapsulation efficiency, and in vitro drug release. Table 3 presents the coded levels of formulation variables and corresponding responses.

Table 3. Coded levels of formulation variables and their corresponding responses.

Run	Entrapment Efficiency (%)	Particle Size (nm)	Cumulative Drug Release (%)
1	85.51	177	62.2
2	84.39	198	70.1
3	91.12	202	78.4
4	92.42	208	63.4
5	91.15	197	73.5
6	92.38	182	76.1
7	78.92	207	70.5
8	89.88	200	71.0
9	81.61	225	77.4
10	86.95	213	66.5
11	83.82	225	64.4
12	90.55	192	73.7
13	92.09	185	75.6
14	88.15	208	72.8
15	90.11	197	72.3

Particle Size Analysis

The particle size of the prepared Letrozole-loaded nanoemulsions ranged from **177 nm to 225 nm** (Table 3), depending on the concentrations of oil and surfactants. Increasing oil content increased droplet size due to enhanced viscosity, while higher surfactant concentrations reduced interfacial tension, producing smaller and more uniform droplets. Optimized emulsification conditions ensured a narrow size distribution favorable for targeted drug delivery.

Encapsulation Efficiency

Encapsulation efficiency ranged from **83.82 % to 93.42 %** (Table 3), indicating efficient entrapment of Letrozole within the nanoemulsion droplets. Higher surfactant and co-surfactant ratios improved solubilization and reduced drug precipitation, enhancing drug retention in the dispersed phase.

In Vitro Drug Release

The in vitro drug release study in phosphate buffer (pH 6.8) revealed cumulative release from **64.4 % to 78.4 %** over 8 hours (Table 3). Formulations with higher surfactant content showed slightly faster release due to improved diffusion, whereas those with higher oil content exhibited sustained release, confirming the role of lipid concentration in controlling Letrozole release kinetics.

Impact of Formulation Variables on Nanoemulsion Characteristics

Design of Experiments (DoE) and ANOVA analysis were used to evaluate the effects of formulation components (Table 4). The linear model for encapsulation efficiency (Response 1) was statistically significant ($F = 5.83$, $p = 0.0124$), with oil (A) and surfactant (B) concentrations as significant factors ($p < 0.05$). The non-significant Lack of Fit ($F = 0.30$, $p = 0.916$) confirmed model adequacy. For particle size (Response 2), the model was significant ($F = 4.24$, $p = 0.0320$), with oil (A) and surfactant (B) having strong influence on droplet formation. Cumulative drug release (Response 3) followed a 2FI model ($F = 6.11$, $p = 0.0113$), where surfactant and co-surfactant interaction significantly affected release behavior ($p = 0.0500$).

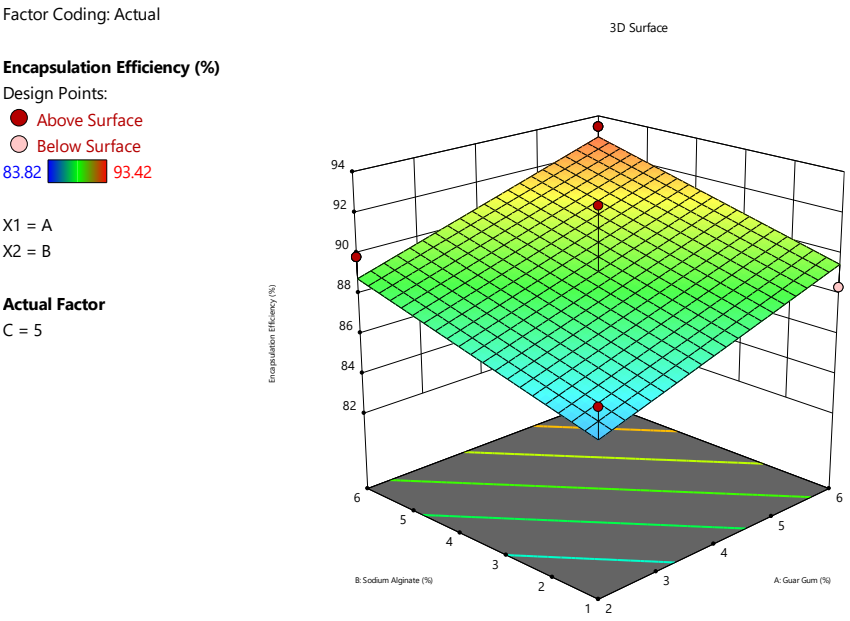

Table 4. ANOVA results for Letrozole-loaded nanoemulsion responses.

Source	Encapsulation Efficiency (F, p)	Particle Size (F, p)	Cumulative Drug Release (F, p)
Model	5.83, 0.0124 (Significant)	4.24, 0.0320 (Significant)	6.11, 0.0113 (Significant)
Lack of Fit	0.30, 0.916 (NS)	0.26, 0.937 (NS)	0.03, 0.9995 (NS)

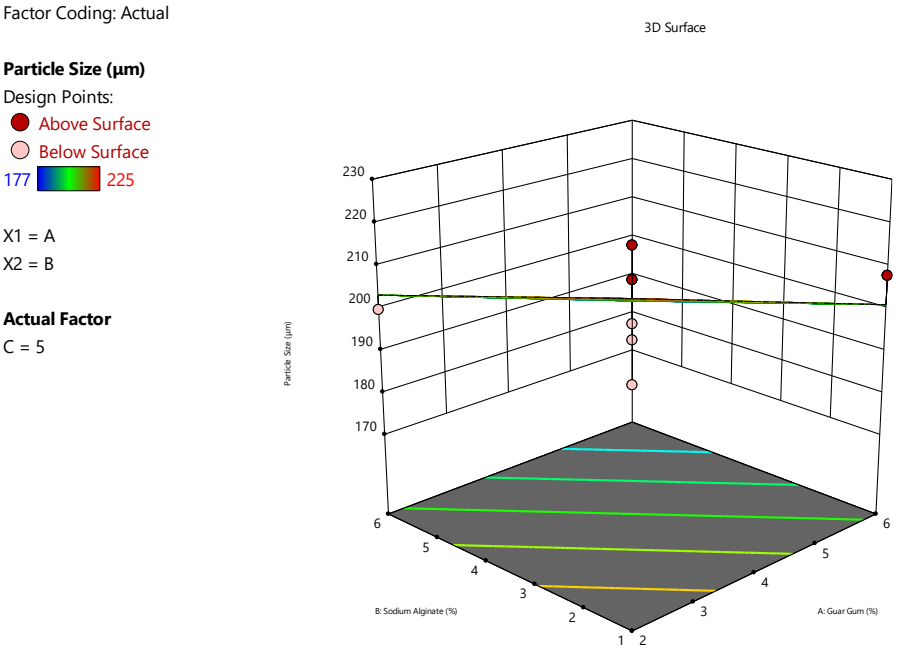

Figure 2. 3D Graph showing the effect of formulation factors on Entrapment Efficiency.

Figure 3. 3D Surface showing the effect of formulation factors on Particle Size.

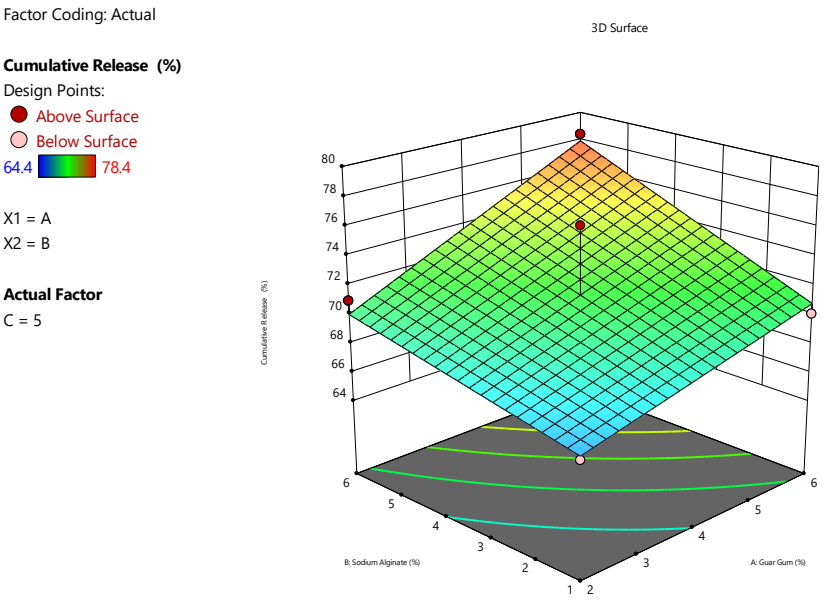

Figure 4. 3D Surface Showing the effect of formulation factors on Drug Release.

Optimization of Letrozole-Loaded Nanoemulsion

Based on DoE, the optimized batch (F-16) achieved maximum encapsulation efficiency with controlled particle size and sustained release. A total of 63 solutions were generated; the formulation with highest desirability (0.920) was selected (Table 6). The optimized concentrations were **Oil 6.00 % v/v, Surfactant 6.00 % v/v**, and **Co-surfactant 4.61 % v/v**, with stirring speed 1500 rpm and solvent volume 10 mL (Table 5). Predicted and experimental results showed excellent correlation, with < 3 % error in all responses.

Table 5. Optimized batch (F-16) composition of Letrozole-loaded nanoemulsion.

Factor	Optimized Value
Oil (Capryol 90)	6.00 % v/v
Surfactant (Tween 80)	6.00 % v/v
Co-surfactant (Transcutol P)	4.61 % v/v
Stirring Speed	1500 rpm
Solvent Volume	10 mL

Table 6. Predicted vs. experimental responses of optimized batch (F-16).

Response	Predicted Value	Experimental Value	% Error
Entrapment Efficiency (%)	92.75	91.85 ± 0.4	0.97 %
Particle Size (nm)	183.45	180.75 ± 1.5	1.47 %
Cumulative Drug Release (%)	78.40	76.25 ± 0.6	2.79 %
Desirability	0.920		

Morphological Analysis

The surface morphology of the **optimized batch (F16)** of Letrozole-loaded nanoemulsion was examined using **Scanning Electron Microscopy (SEM)**. The SEM micrograph (Figure 5) revealed uniformly distributed, spherical particles with a smooth surface and absence of aggregation, confirming homogeneity and good physical stability of the formulation.

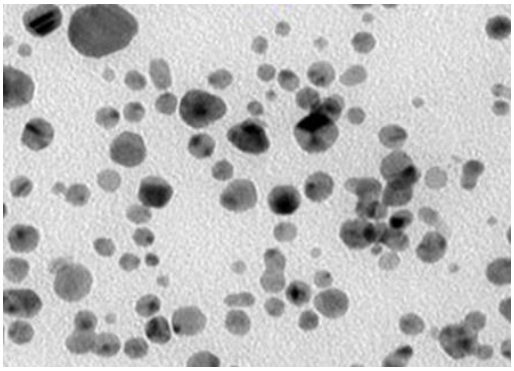

Figure 5. SEM image of the optimized Letrozole-loaded Nanoemulsion

CONCLUSION

The present study successfully designed and optimized a Letrozole-loaded nanoemulsion for targeted breast cancer therapy using the Box–Behnken statistical design. The formulation parameters oil, surfactant, and co-surfactant concentrations significantly influenced particle size, encapsulation efficiency, and drug release behavior. The optimized nanoemulsion demonstrated a nanosized droplet range (approximately 180 nm), high encapsulation efficiency (over 91%), and sustained drug release up to 12 hours, confirming its potential for prolonged therapeutic action. Morphological analysis revealed spherical, uniform, and stable droplets, indicating good formulation stability. The results collectively suggest that nanoemulsion-based delivery of Letrozole can enhance its solubility, bioavailability, and therapeutic efficacy while minimizing systemic side effects. Thus, the developed Letrozole-loaded nanoemulsion represents a promising nanocarrier system for improving the effectiveness of hormone-dependent breast cancer treatment.

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Conflict of Interest

No conflict of interest.

Funding

NIL

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