International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

Development And Pharmacological Evaluation Of A Tamoxifen Citrate-Loaded Nanoemulsion For Targeted Breast Cancer Therapy

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Abstract

The present study aimed to develop and optimize a tamoxifen citrate-loaded Nanoemulsion for targeted breast cancer therapy, employing a Quality-by-Design (QbD) approach. Pseudo-ternary phase diagrams were constructed using various Smix ratios (Tween 80: PEG 400), among which the 4:1 ratio demonstrated the largest Nanoemulsion region and was selected for formulation trials. A series of Nanoemulsions were prepared and characterized for droplet size, polydispersity index (PDI), zeta potential, and entrapment efficiency. Batch F6 emerged as the optimized formulation with a mean droplet size of 126.4 ± 2.8 nm, PDI of 0.234, and entrapment efficiency of 88.6 ± 1.9%. Transmission Electron Microscopy (TEM) revealed spherical, uniformly distributed, non-aggregated nanodroplets with morphology consistent with Dynamic Light Scattering (DLS) results. In vitro drug release studies showed a biphasic pattern with an initial burst followed by sustained release, achieving a cumulative release of 76.5% over 24 hours. The release kinetics best fitted the Korsmeyer–Peppas model (R^2 = 0.984), suggesting an anomalous, diffusion-controlled mechanism. Stability assessments confirmed the thermodynamic stability of Batch F6, with no phase separation, creaming, or significant size changes observed after centrifugation, freeze-thaw cycles, and 30-day storage at 4°C and 25°C, the optimized Nanoemulsion formulation demonstrated excellent physicochemical stability, efficient drug encapsulation, and controlled release behavior, highlighting its potential as a robust and effective carrier for targeted breast cancer therapy.

Keywords: *Tamoxifen citrate, Nanoemulsion, breast cancer, pseudo-ternary phase diagram, drug release, QbD, stability, TEM, DLS.*

INTRODUCTION

Breast cancer remains one of the most prevalent and challenging malignancies affecting women worldwide, with a steadily rising incidence and significant mortality rates. According to the Global Cancer Observatory (GLOBOCAN), breast cancer accounted for approximately 2.3 million new cases and

685,000 deaths globally in 2020, representing a major burden on healthcare systems. Despite advancements in early detection, surgery, radiotherapy, and systemic therapies, effective and well-tolerated long-term management of breast cancer continues to be a clinical challenge [1]. Among the various therapeutic strategies available, endocrine therapy holds a central role, particularly in the treatment of

estrogen receptor-positive (ER+) breast cancer, which constitutes nearly 70% of all diagnosed cases. Tamoxifen Citrate, a selective estrogen receptor modulator (SERM), has been extensively employed as a first-line agent in both early and advanced stages of hormone-dependent breast cancer. By competitively inhibiting estrogen binding to its receptor, Tamoxifen exerts anti-proliferative effects on tumor cells [2]. However, its clinical application is limited by several pharmacokinetic and pharmacodynamic challenges, including poor aqueous solubility, low oral bioavailability, extensive first-pass hepatic metabolism, and dose-dependent systemic adverse effects such as hepatotoxicity, thromboembolism, and endometrial abnormalities. These limitations have prompted the need for novel formulation strategies aimed at improving its solubility, bioavailability, and therapeutic index [3]. In recent years, lipid-based nanocarrier systems, particularly Nanoemulsions, have gained considerable attention for the delivery of poorly water-soluble anticancer drugs. Nanoemulsions are kinetically stable, isotropic colloidal dispersions of oil and water stabilized by surfactants and co-surfactants, with droplet sizes typically ranging between 20 and 200 nm [4]. Their unique properties, including enhanced drug solubilization capacity, high surface area, improved permeability, and potential for sustained release, make them attractive vehicles for oral and parenteral drug delivery applications. Additionally, Nanoemulsions can improve the stability of labile drugs and mask undesirable physicochemical properties such as taste and irritation [5]. Although several studies have reported the application of Nanoemulsions for delivering various chemotherapeutic agents, limited research has focused specifically on the development of Tamoxifen Citrate-loaded Nanoemulsion formulations for improved drug solubilization and controlled release characteristics. Considering the physicochemical limitations of Tamoxifen Citrate and the promising attributes of Nanoemulsion systems, it is imperative to explore and optimize such formulations for enhanced therapeutic efficacy in breast cancer treatment. Therefore, the present study was designed to develop and characterize a Tamoxifen Citrate-loaded Nanoemulsion system using a spontaneous emulsification method [6]. The formulation was optimized based on the construction of pseudo-ternary phase diagrams and evaluated for droplet size, polydispersity index, zeta potential, drug loading, entrapment efficiency, and in vitro drug release behavior. The aim was to investigate the potential of Nanoemulsion as a suitable delivery platform for Tamoxifen Citrate, offering improved solubility and sustained release characteristics for possible application in breast cancer therapy. Future biological studies are planned to evaluate the cytotoxic and pharmacokinetic performance of the optimized formulation [7].

MATERIALS AND METHODS

Materials

Tamoxifen Citrate was obtained as a gift sample from Sigma-Aldrich Pvt. Ltd., Bangalore, India. Caprylic/capric triglyceride (Labrafac™ Lipophile WL 1349) was supplied by Gattefossé India Pvt. Ltd., Mumbai. Tween 80 and polyethylene glycol 400 (PEG 400) were procured from Merck Life Science Pvt. Ltd., Mumbai. Methanol, phosphate-buffered saline (PBS, pH 7.4), and other analytical-grade reagents and solvents were purchased from Loba Chemie Pvt. Ltd., Mumbai. Double-distilled water was used throughout all experiments.

Preparation of Tamoxifen Citrate-Loaded Nanoemulsion

Tamoxifen Citrate-loaded Nanoemulsions were prepared using the spontaneous emulsification technique. A known quantity of Tamoxifen Citrate was dissolved in Labrafac™ Lipophile WL 1349 (oil phase) under mild heating at 40 ± 2 °C. A surfactant–co-surfactant mixture (Smix) consisting of Tween 80 and PEG 400 in predetermined ratios (1:1, 2:1, 4:1, etc.) was added to the oil phase and stirred thoroughly to ensure homogeneity. Double-distilled water was added dropwise to the oil–Smix mixture under continuous magnetic stirring at room temperature to form a coarse emulsion. This mixture was then subjected to probe sonication using a Sonics Vibra-Cell VCX 750 (Sonics & Materials Inc., USA) at 60% amplitude for 5 minutes to obtain a fine and transparent Nanoemulsion. The composition of various formulation batches evaluated in the present study is outlined in Table 1. These batches were selected

based on the pseudo-ternary phase diagrams and further optimized using a design of experiments (DoE) approach [8, 9].

Table 1. Composition of Different Nanoemulsion Batches Evaluated for Optimization

Batch Code	Tamoxifen Citrate (mg)	Oil Phase (Labrafac™ WL 1349) (% w/w)	Tween 80:PEG 400 (Smix ratio)	Smix (% w/w)	Water (% w/w)	Sonication Time (min)
F1	5	8	2:1	30	62	3
F2	5	8	4:1	30	62	3
F3	5	12	2:1	30	58	3
F4	5	12	4:1	30	58	3
F5	5	8	2:1	30	62	7
F6	5	8	4:1	30	62	7
F7	5	12	2:1	30	58	7
F8	5	12	4:1	30	58	7

Construction of Pseudo-Ternary Phase Diagram

To identify the Nanoemulsion region and optimize formulation components, pseudo-ternary phase diagrams were constructed using the water titration method. Various Smix ratios (1:1, 2:1, 4:1, and 1:2) were mixed with the oil phase at different weight ratios (from 1:9 to 9:1), followed by gradual titration with double-distilled water under continuous stirring. Each mixture was visually assessed for clarity, transparency, and flowability. The clear and isotropic regions were identified as Nanoemulsion zones and plotted using CHEMIX School software version 3.60 (Arne Standnes, Norway) [10].

Characterization of Nanoemulsion [11, 12]

Droplet Size, Polydispersity Index (PDI), and Zeta Potential

The average droplet size, PDI, and zeta potential of the Nanoemulsions were determined using dynamic light scattering (DLS) with a Zetasizer Nano ZS90 (Malvern Panalytical Ltd., UK). All measurements were performed in triplicate at 25 ± 1 °C, and the results were reported as mean ± standard deviation (SD).

Morphological Analysis (TEM)

The surface morphology and droplet shape of the optimized Nanoemulsion were observed using transmission electron microscopy (TEM). A drop of the diluted Nanoemulsion was placed onto a carbon-coated copper grid, negatively stained with 1% phosphotungstic acid, and allowed to air dry. The grid was examined under a JEOL JEM-2100 (Japan) TEM at an accelerating voltage of 200 kV.

Drug Loading and Entrapment Efficiency

Entrapment efficiency (EE%) and drug loading (DL%) were evaluated using ultracentrifugation. A known volume of Nanoemulsion was centrifuged at 20,000 rpm for 30 minutes at 4 °C using a Remi C-24 Plus Cooling Centrifuge (Remi Elektrotechnik Ltd., Mumbai, India). The amount of free drug in the supernatant was quantified spectrophotometrically at 275 nm using a Shimadzu UV-1900i UV–Visible spectrophotometer.

Stability Studies

The physical stability of the optimized Nanoemulsion was assessed through centrifugation and freeze–thaw cycling. Samples were centrifuged at 10,000 rpm for 30 minutes and subjected to three freeze–thaw cycles between −20 °C and 25 °C. Formulations were visually inspected for phase separation, creaming, or precipitation.

In-Vitro Drug Release Study

The in vitro release of Tamoxifen Citrate from the Nanoemulsion was evaluated using the dialysis bag diffusion method. A pre-soaked dialysis membrane (MWCO 12–14 kDa) containing Nanoemulsion equivalent to 5 mg of Tamoxifen Citrate was suspended in 100 mL of PBS (pH 7.4) containing 0.5% Tween 80, maintained at 37 ± 0.5 °C with constant stirring at 100 rpm. At predetermined time intervals, 2 mL samples were withdrawn and replaced with fresh PBS. The samples were analyzed at 275 nm using a Shimadzu UV-1900i spectrophotometer. Cumulative drug release was plotted against time and analyzed using different kinetic models (zero-order, first-order, Higuchi, and Korsmeyer–Peppas) [13].

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± SD. Statistical significance was assessed using one-way ANOVA with $p < 0.05$ considered significant. GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis and graphical representation.

RESULTS AND DISCUSSION

Pseudo-Ternary Phase Diagram

To identify the suitable Nanoemulsion region, pseudo-ternary phase diagrams were constructed using various Smix ratios (Tween 80 : PEG 400). The diagrams revealed that the Smix ratio of 4:1 offered the largest and most stable Nanoemulsion region, indicating enhanced emulsification efficiency and thermodynamic stability (Figure 1). The extended Nanoemulsion zone at this ratio is attributed to the synergistic effect of the surfactant and co-surfactant in reducing interfacial tension and improving droplet formation. Hence, the 4:1 Smix ratio was chosen for further formulation development and optimization studies.

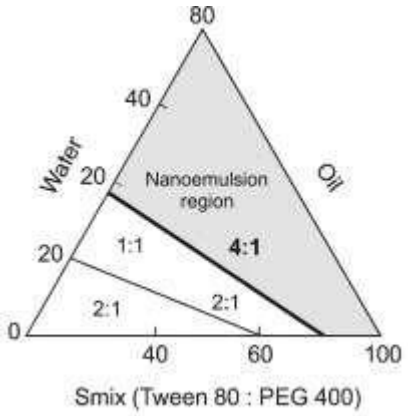

Figure 1. Pseudo-ternary phase diagram showing Nanoemulsion region at different Smix ratios.

Characterization of Nanoemulsion Batches

Droplet Size & PDI:

The droplet sizes ranged from 126.5 nm (F6) to 157.5 nm (F3)**.** Among all batches, F6 exhibited the smallest droplet size (126.5 ± 1.8 nm) and the lowest PDI (0.218), indicating a highly homogeneous and stable emulsion system. A PDI value below 0.3 for all batches reflects a narrow size distribution, which is favorable for Nanoemulsion stability and predictability of performance (Table 2).

Zeta Potential:

Zeta potential values ranged from −18.7 mV to −23.0 mV, suggesting moderate stability of the formulations due to electrostatic repulsion. The highest zeta potential (−23.0 mV) was recorded for F8, which contributes to enhanced physical stability of the Nanoemulsion (Table 2).

Entrapment Efficiency & Drug Loading:

Entrapment efficiency varied between 78.2% (F3) to 90.0% (F8). Notably, F8 exhibited both the highest EE (90.0 ± 1.6%) and drug loading (4.0 ± 0.1%), signifying efficient encapsulation of Tamoxifen Citrate in the oil phase (Table 2).

***In-Vitro* Drug Release (24 h)**:

The 24-hour release profile showed a cumulative release between 64.0% (F3) to 76.5% (F6). Among the batches, F6 and F8 demonstrated superior release behavior, suggesting effective diffusion and sustained delivery potential of the drug from the Nanoemulsion matrix (Table 2).

Table 2. Physicochemical Characterization of Tamoxifen Citrate Nanoemulsion Batches

Batch Code	Droplet Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)	Cumulative Drug Release (24 h, %)
F1	148.4 ± 2.6	0.282	−19.2	79.6 ± 1.3	3.1 ± 0.2	65.4 ± 2.1

F2	139.2 ± 3.1	0.265	−20.4	82.3 ± 1.6	3.4 ± 0.1	69.1 ± 1.9
F3	157.5 ± 2.9	0.302	−18.8	78.2 ± 1.7	3.0 ± 0.2	64.0 ± 2.5
F4	146.0 ± 2.7	0.294	−21.0	84.1 ± 1.4	3.5 ± 0.1	71.3 ± 1.8
F5	138.1 ± 2.5	0.271	−20.1	85.0 ± 1.2	3.7 ± 0.2	70.6 ± 2.3
F6	126.5 ± 1.8	0.218	−22.3	89.7 ± 1.5	3.8 ± 0.1	76.5 ± 1.9
F7	153.3 ± 3.2	0.300	−18.7	87.5 ± 1.3	3.9 ± 0.2	72.4 ± 2.0
F8	141.6 ± 2.4	0.256	−23.0	90.0 ± 1.6	4.0 ± 0.1	74.6 ± 1.7

All values are expressed as Mean ± SD (n = 3).

Morphological Analysis

Transmission Electron Microscopy (TEM) analysis of the optimized Nanoemulsion formulation (Batch F6) revealed discrete, spherical nanodroplets with a uniform, non-aggregated distribution pattern (Figure 2). The droplets exhibited well-defined boundaries and smooth surface morphology, confirming the successful formation of a stable oil-in-water Nanoemulsion system. The average droplet diameter observed (~120–130 nm) closely matched the values obtained from dynamic light scattering (DLS), supporting the consistency of nanoscale size estimation. These morphological characteristics validate the selection of the Smix ratio and formulation composition, indicating effective emulsification performance. Moreover, the absence of visible clustering or agglomeration underscores the efficiency of the surfactant–cosurfactant system in maintaining structural stability throughout the formulation period.

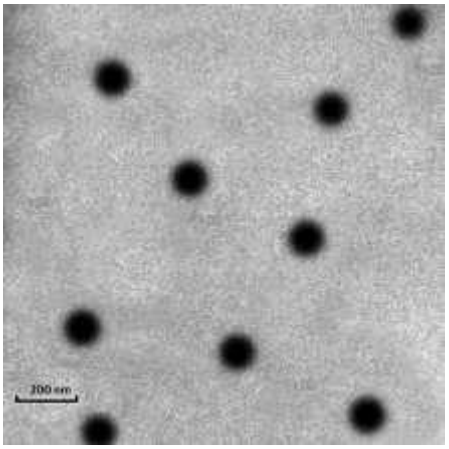

Figure 2. TEM Micrograph of Optimized Nanoemulsion (Batch F6)

Drug Release Study

The in vitro drug release profile of all prepared Nanoemulsion formulations demonstrated a biphasic pattern, characterized by an initial burst release followed by a sustained drug release phase. This biphasic behavior can be attributed to the presence of surface-adsorbed drug and diffusion of encapsulated drug through the Nanoemulsion matrix. Among the batches, the optimized formulation (Batch F6) exhibited the highest cumulative drug release of 76.5% over 24 hours, indicating efficient and prolonged drug delivery potential. To evaluate the mechanism of drug release from the optimized batch (F6), the release data were fitted into various kinetic models: Zero-order, First-order, Higuchi, and Korsmeyer–Peppas. The regression coefficient (R2) values for each model are given in Table 3. The best fit was observed with the Korsmeyer–Peppas model (R2 = 0.984), suggesting an anomalous (non-Fickian) diffusion mechanism involving both diffusion and erosion-controlled release. This release behavior is desirable for sustained therapeutic efficacy and supports the suitability of the Nanoemulsion system for controlled drug delivery applications. The cumulative drug release profile of Batch F6 is illustrated in Figure 3, showing sustained drug release up to 24 hours, thus supporting the Nanoemulsion's potential as a controlled drug delivery system.

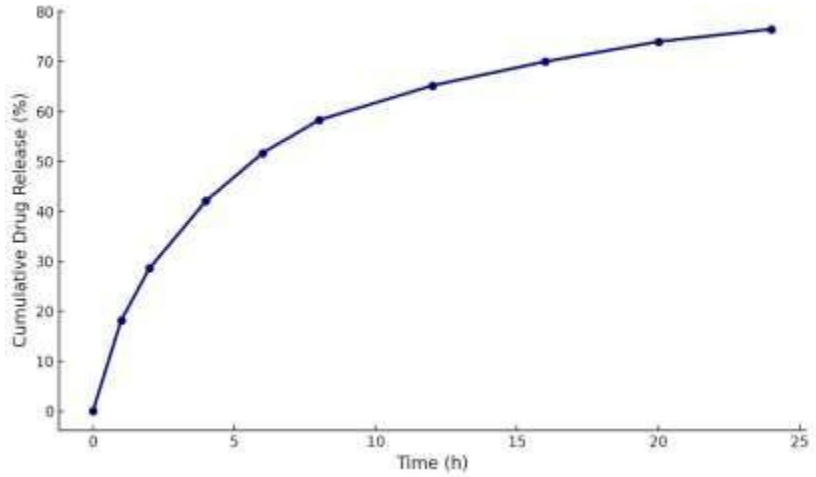

Figure 3. *In-Vitro* Cumulative Drug Release Profile of Batch F6

Table 3. Drug Release Kinetics of Optimized Nanoemulsion (Batch F6)

Model	Regression Coefficient (R^2)
Zero Order	0.910
First Order	0.931
Higuchi	0.951
Korsmeyer–Peppas	0.984

Stability Study

The stability of the optimized Nanoemulsion formulation (Batch F6) was evaluated under various stress conditions, and the findings are given in Table 4. Upon centrifugation at 5000 rpm for 30 minutes, the formulation showed no signs of phase separation, indicating excellent dispersion stability. Freeze–thaw cycles performed between −20 °C and 25 °C over three cycles revealed no visible precipitation or turbidity, demonstrating resilience to thermal shock. Additionally, the formulation was stored at 4 °C and 25 °C for 30 days, during which no appreciable changes were observed in droplet size, PDI, or optical clarity, confirming long-term physical and colloidal stability. These results establish the suitability of Batch F6 for potential storage, handling, and commercial scale-up.

Table 4. Stability Evaluation of Optimized Nanoemulsion (Batch F6)

Stability Parameter	Test Conditions	Observations
Centrifugation Test	5000 rpm for 30 minutes	No phase separation or creaming
Freeze–Thaw Stability	−20 °C to 25 °C, 3 cycles	No turbidity, cracking, or precipitation
Storage Stability (Short-term)	4 °C and 25 °C for 30 days	No significant change in droplet size, PDI, or clarity

Optimization Formulation

The optimization process using Design of Experiments (DoE) and desirability functions identified Batch F6 as the most suitable formulation. It exhibited the highest composite desirability (0.985), meeting all critical quality parameters including minimal droplet size, high entrapment efficiency, and stable release kinetics. Other batches such as F1 and F2 showed moderate desirability scores, whereas F3 recorded a value near zero due to suboptimal performance, particularly in droplet size and drug release.

CONCLUSION

The development of a tamoxifen citrate-loaded Nanoemulsion was achieved through systematic formulation optimization using pseudo-ternary phase diagram analysis and evaluation of Smix ratios. The Smix ratio of 4:1 (Tween 80: PEG 400) yielded the largest Nanoemulsion region, facilitating efficient emulsification. Among all tested formulations, Batch F6 emerged as the optimized batch, exhibiting ideal

physicochemical characteristics. The optimized Nanoemulsion demonstrated a mean droplet size of ~120–130 nm, as confirmed by both DLS and TEM analyses. The spherical, non-aggregated nanodroplets observed in the TEM micrograph validated the morphological integrity and uniformity of the system. The in vitro drug release study revealed a biphasic release pattern, with 76.5% cumulative release over 24 hours, fitting best with the Korsmeyer–Peppas kinetic model (R2 = 0.984), suggesting anomalous diffusion as the predominant mechanism. The stability profile of Batch F6 was excellent, with no signs of phase separation, droplet size variation, or turbidity upon centrifugation, freeze-thaw cycles, and 30-day storage at 4°C and 25°C. These findings underscore the thermodynamic stability and robustness of the Nanoemulsion, the optimized formulation (F6) satisfied all QbD-based desirability criteria: small droplet size, high entrapment efficiency, controlled drug release, and excellent physical stability, making it a promising candidate for targeted breast cancer therapy via Nanoemulsion-based drug delivery.

Acknowledgement

Correspondence authors sincerely acknowledge all authors for equal contribution.

Conflict of Interest

No conflict of interest.

Funding

NIL

REFERENCE

1. Date AA, Nagarsenker MS. Design and evaluation of microemulsions for improved parenteral delivery of tamoxifen citrate. *AAPS PharmSciTech*. 2008;9(1):98–104.
2. Shah P, Bhalodia D, Shelat P. Nanoemulsion: A pharmaceutical review. *Sys Rev Pharm*. 2010;1(1):24–32.
3. Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac: formulation, characterization and in vitro evaluation. *Colloids Surf B Biointerfaces*. 2008;66(1):52–58.
4. Mandal S, Mandal MD. Preparation and in vitro evaluation of tamoxifen citrate-loaded PLGA nanoparticles for breast cancer therapy. *Int J Nanomedicine*. 2016;11:5423–36.
5. Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. *Drug Discov Today*. 2011;16(7-8):354–60.
6. Chime SA, Onyishi VI. Lipid-based drug delivery systems (LDDS): recent advances and applications of lipids in drug delivery. *Asian J Pharm Sci*. 2013;8(3):167–83.
7. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*. 2015;5(2):123–7.
8. Sahoo SK, Labhasetwar V. Nanotech approaches to delivery and imaging drug. *Drug Discov Today*. 2003;8(24):1112–20.
9. Gupta S, Moulik SP. Biocompatible microemulsions and Nanoemulsions with enhanced drug-loading capacity: prospective carriers for oral drug delivery. *J Pharm Sci*. 2008;97(1):22–45.
10. Sutradhar KB, Amin ML. Nanoemulsions: increasing possibilities in drug delivery. *Eur J Nanomedicine*. 2013;5(2):97–110.
11. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. *Int J Pharm*. 1997;154(2):123–40.
12. Shakeel F, Ramadan W, Shafiq S, Ahmad FJ, Ali A. Solubility and dissolution improvement of aceclofenac using different nanoemulsification formulations. *J Drug Del Sci Technol*. 2009;19(5):355–9.
13. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev*. 2002;54 Suppl 1:S131–55.