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Development, Optimization, and Characterization of Nanoengineered Drug Delivery Platforms for Tamoxifen: A Next-Generation Strategy for Sustained and Targeted Release in Hormone-Responsive Breast Cancer Treatment

Tej Pratap Singh[1], Anita. A[2], Komal Kriti[*3], Ashutosh Pathak[4], Nikunj Solanki[5], Sonal Solanki[6], Sabnam Nargis[7], Butchi Raju Akondi[8],

[1]Department of Pharmaceutics, Satyadeo College of Pharmacy, Borsiya, Fadanpur, Ghazipur, Uttar Pradesh – 233001.

[2]Dayananda Sagar University, college of Pharmaceutical Sciences Bangalore 562112.

[*3]Department of Pharmaceutical Sciences, Dhanbad College of Pharmacy and Research Institute, Near BBMKU, Shakti Nagar, Nag Nagar, Dhanbad, Jharkhand – 826004, India.

[4]Department of Pharmacy Practice, Teerthanker Mahaveer College of Pharmacy, Teerthanker

Mahaveer University, Moradabad UP, India Pin- 244001.

[5,6]Department of Pharmaceutics, HSBPVT's, Group of Institutions, Faculty of Pharmacy, Kashti, Ta- Shrigonda, Ahilyanagar, Maharastra. Pin - 414701.

[7]Department of Pharmaceutical Chemistry, Royal School of Pharmacy, The Assam Royal Global University, Guwahati, Assam Pincode-781035.

[8]Department of Clinical Pharmacy and Pharmacology, Ibn Sina National College for Medical Studies, Jeddah, 22421.

***Corresponding Author:**

Komal Kriti

Email ID: kk26061993@gmail.com

ABSTRACT

Tamoxifen, a selective estrogen receptor modulator, is widely used in the treatment of estrogen receptor-positive (ER+) breast cancer. However, its poor aqueous solubility and bioavailability limit its therapeutic effectiveness. This study aimed to develop Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) to enhance its solubility, bioaccessibility, and cytotoxic potential. The formulations were optimized using solid and liquid lipids, surfactants, and stabilizers, ensuring high encapsulation efficiency (87–91%) and a controlled release profile. Particle size analysis showed that freshly prepared NDDPs were in the 41–43 nm range, while lyophilized formulations exhibited a slight increase in size (182–184 nm) but retained stability. In vitro drug release under simulated intestinal conditions followed a biphasic pattern, with sustained release up to 360 minutes (93.35–93.45%), supporting prolonged drug availability. Bioaccessibility studies demonstrated a 5-fold increase in micellar solubilization compared to pure Tamoxifen. Cytotoxicity evaluation using MCF-7 breast cancer cells revealed that NDDP-3 exhibited significantly enhanced cytotoxic effects compared to free Tamoxifen, reducing cell viability to 4.18% at lower concentrations. The results highlight the potential of lipid-based nanocarrier systems to enhance Tamoxifen's therapeutic efficacy by improving drug delivery, bioavailability, and intracellular retention.

Keywords: *Tamoxifen, Nanoengineered Drug Delivery Platforms, Chemotherapeutic agents, lipid based nanocarriers, Bioaccessibility enhancement Nanostructured Carriers, Oral drug delivery.*

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1. INTRODUCTION

Breast cancer is one of the most prevalent malignancies worldwide, with estrogen receptor-positive (ER+) breast cancer constituting a significant proportion of cases. Tamoxifen, a selective estrogen receptor modulator (SERM), has remained the gold standard for the treatment and prevention of ER+ breast cancer due to its ability to block estrogen-driven tumor growth. However, despite its clinical success, the therapeutic potential of Tamoxifen is significantly limited by its poor aqueous solubility, low bioavailability, and suboptimal pharmacokinetics. These drawbacks often lead to inconsistent therapeutic outcomes, requiring higher doses that may result in systemic toxicity and adverse side effects such as thromboembolic events and endometrial hyperplasia. Hence, there is an urgent need for novel drug delivery strategies that can enhance the solubility, bioavailability, and therapeutic efficacy of Tamoxifen while minimizing systemic side effects (Dunn et al., 2004; Jones & Baylin, 2007; Peer et al., 2020; Pérez-Herrero & Fernández-Medarde, 2015).

Tamoxifen is a lipophilic compound (log P ~6.3) with poor water solubility (~0.4 mg/L), which leads to erratic absorption and low oral bioavailability (~20%). Following oral administration, Tamoxifen undergoes extensive first-pass metabolism in the liver, resulting in the formation of active metabolites, such as 4-hydroxy-Tamoxifen and endoxifen, which contribute to its therapeutic effect. However, the low bioavailability of the parent drug and its reliance on metabolic activation often lead to variability in patient response. Additionally, Tamoxifen's high plasma protein binding (~99%) further restricts its free drug concentration, limiting its ability to effectively reach tumor cells. Another challenge with conventional Tamoxifen therapy is its dose-dependent toxicity (Beloqui et al., 2016; Dingler & Gohla, 2002; Iqbal et al., 2012; Joshi et al., 2019; Punu et al., 2023). At higher doses, systemic exposure to Tamoxifen can lead to severe adverse effects, including cardiovascular complications, hepatic toxicity, and an increased risk of secondary malignancies such as endometrial cancer. These drawbacks necessitate the development of nanotechnology-based drug delivery systems that can improve Tamoxifen's pharmacokinetic properties, ensuring controlled release, targeted delivery, and enhanced therapeutic efficacy (Montecucco & Biamonti, 2007; Montecucco et al., 2015; Slevin, 1991).

Nanotechnology has emerged as a promising approach to overcome the solubility and bioavailability limitations of poorly water-soluble drugs like Tamoxifen. Lipid-based nanocarriers, including nanoemulsions, liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), have gained significant attention due to their ability to enhance drug solubilization, improve cellular uptake, and provide controlled drug release. These systems mimic natural lipid absorption pathways, facilitating lymphatic transport, thereby bypassing first-pass metabolism and improving oral bioavailability. Among lipid-based systems, Nanoengineered Drug Delivery Platforms (NDDPs) represent a novel approach for encapsulating Tamoxifen in a stable lipid matrix, allowing for higher drug loading, prolonged circulation, and improved bioaccessibility. Lipid nanocarriers facilitate the self-emulsification of lipophilic drugs in gastrointestinal fluids, promoting enhanced solubilization and micellar incorporation, leading to greater drug absorption across intestinal membranes. Additionally, these systems can be tailored to provide site-specific delivery, minimizing off-target toxicity and enhancing drug accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect (Beloqui et al., 2016; Joshi et al., 2019; Khan, 2010; Naseri et al., 2015; Peer et al., 2020; Pérez-Herrero & Fernández-Medarde, 2015).

Given the limitations of conventional Tamoxifen therapy and the potential of lipid nanocarriers to improve drug delivery, this study aims to develop and evaluate Nanoengineered Drug Delivery Platforms (NDDPs) for Tamoxifen, focusing on their ability to enhance solubility, bioaccessibility, and anticancer efficacy. The study explores key formulation parameters, including particle size, encapsulation efficiency, zeta potential, in vitro drug release, bioaccessibility, and cytotoxicity, to assess the suitability of NDDPs as a novel Tamoxifen delivery system (Griffith, 1981; Ibrahim et al., 2021; Naseri et al., 2015; Stevens, 2006). The hypothesis is that Tamoxifen-loaded NDDPs will exhibit superior solubility, bioavailability, and sustained drug release compared to free Tamoxifen, leading to enhanced anticancer efficacy with reduced toxicity. he primary objective of this study was to formulate and optimize Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) using a lipid-based system to enhance solubility, bioavailability, and therapeutic efficacy. The study aimed to characterize the physicochemical properties of NDDPs, including particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and drug loading capacity, to ensure uniformity, stability, and effective drug entrapment. Additionally, the in vitro drug release behavior of NDDPs was evaluated under simulated gastrointestinal conditions, assessing the sustained release potential and suitability for oral administration. The study further investigated the bioaccessibility of Tamoxifen from NDDPs and compared it with free Tamoxifen to determine the extent of drug solubilization and potential absorption enhancement. Finally, the cytotoxic effects of NDDPs were assessed in MCF-7 breast cancer cells using the MTT assay, with a comparative analysis against free Tamoxifen to evaluate the potential for enhanced anticancer activity. These objectives collectively aimed to establish lipid-based NDDPs as a promising drug delivery system for Tamoxifen, addressing solubility limitations and improving therapeutic outcomes in estrogen receptor-positive breast cancer treatment. The successful development of Tamoxifen-loaded NDDPs can potentially revolutionize breast cancer treatment by addressing the solubility, bioavailability, and toxicity challenges associated with conventional formulations. By enhancing drug absorption and ensuring sustained release, these lipid nanocarriers could significantly reduce dosing frequency, thereby improving patient compliance and therapeutic outcomes. Moreover, this study will provide valuable insights into the role of nanocarrier-based drug delivery in breast cancer therapy, paving the way for

further clinical translation and pharmaceutical development of nanotechnology-driven oral formulations for anticancer drugs.

2. MATERIAL AND METHODS

Drugs, chemicals and allied reagents

The excipients and active pharmaceutical ingredients utilized in the formulation were selected based on their biocompatibility, stability, and functional properties in the development of nanoengineered drug delivery platforms for Tamoxifen. Medium-chain triglyceride (MCT) and Imwitor 900 K, commonly used lipid components for nanoformulations, were obtained as free samples from BASF SE, Ludwigshafen, Germany. These lipids are known for their ability to enhance drug solubility, improve bioavailability, and provide controlled release in lipid-based delivery systems. Lipoid SPC-3, a high-purity soybean phosphatidylcholine, was sourced from Avanti Polar Lipids, Inc., Alabaster, Alabama, USA. Phosphatidylcholine is widely used in lipid-based formulations due to its amphiphilic nature, biocompatibility, and role in forming stable nanoparticle structures, such as liposomes and nanostructured lipid carriers. This excipient plays a critical role in stabilizing the Tamoxifen-loaded nanoparticles, ensuring uniform dispersion and effective drug encapsulation. Tween 80 (Polysorbate 80) and Span 20, well-established non-ionic surfactants, were procured from Sigma-Aldrich, India. These surfactants were incorporated into the formulation to enhance emulsification, reduce interfacial tension, and improve the stability of the developed Nano formulation. Tween 80 is frequently used in drug delivery systems due to its ability to improve cellular uptake and facilitate sustained drug release, while Span 20 contributes to the stabilization of lipid-based nanocarriers. The active pharmaceutical ingredient, Tamoxifen, a selective estrogen receptor modulator (SERM) used for the treatment of estrogen receptor-positive (ER+) breast cancer, was obtained from Sigma-Aldrich, Madrid, Spain. This anticancer agent has limited aqueous solubility, necessitating its incorporation into advanced drug delivery systems such as nanoengineered formulations to improve its pharmacokinetics and therapeutic efficacy. All other chemicals, solvents, and reagents used in the study, including those required for drug encapsulation, characterization, in vitro assays, and release kinetics, were of analytical grade and were procured from certified suppliers to ensure consistency and reliability in experimental procedures. High-performance liquid chromatography (HPLC)-grade solvents were used for analytical estimations to maintain accuracy and reproducibility.

Fabrication of Tamoxifen loaded Nanoengineered Drug Delivery Platforms

The Nanoengineered Drug Delivery Platforms (NDDPs) were prepared using the hot homogenization followed by ultrasonication method to ensure uniform particle size and high encapsulation efficiency (Junyaprasert et al., 2009). Initially, the lipid phase was prepared by accurately weighing the required amounts of solid lipid (Imwitor 900 K) and liquid lipid (Medium-chain triglycerides, MCT) as per Table 1. These components were melted at 70 ± 2°C in a water bath. For the Tamoxifen-loaded formulations (NDDP-1 to NDDP-3), the predetermined amount of Tamoxifen was dissolved in the molten lipid phase under continuous stirring at 500 rpm for 10 minutes, ensuring uniform drug distribution. Simultaneously, the aqueous phase was prepared by dissolving Tween 80, Lecithin, and Span 20 in deionized water, preheated to 70 ± 2°C to maintain compatibility with the lipid phase. The mixture was stirred at 800 rpm until a clear solution was obtained. The hot lipid phase was then added dropwise into the preheated aqueous phase under high-speed homogenization at 12,000 rpm for 5 minutes using a high-shear homogenizer (T25 Ultra-Turrax, IKA), resulting in a coarse emulsion. This emulsion was then subjected to ultrasonication (50% amplitude, 5 minutes, pulse mode) using a probe sonicator (QSonica, USA) to reduce the particle size and obtain a nanoemulsion. The temperature was maintained at 70 ± 2°C throughout the process to prevent premature lipid crystallization. Following sonication, the nanoemulsion was allowed to cool gradually to room temperature (25°C) under gentle stirring at 300 rpm, leading to the solidification of the lipid matrix and formation of Nanoengineered Drug Delivery Platforms (B-NDDP and NDDPs 1–3). The resulting nanosuspension was purified by centrifugation at 15,000 rpm for 30 minutes at 4°C, ensuring the removal of any unencapsulated drug and excess surfactants. The obtained pellet was washed twice with deionized water, redispersed, and subjected to freeze-drying (Labconco, USA) to obtain a dry, free-flowing powder. The final dried nanoparticles were stored in air-tight containers at 4°C for further characterization and evaluation (Elmowafy & Al-Sanea, 2021; Junyaprasert et al., 2009).

Table 1. Formulation of Nanoengineered Drug Delivery Platforms containing blank and Tamoxifen

Formulation	Solid Lipid (%)	Liquid Lipid (%)	Tween 80 (%)	Lecithin (%)	Span 20 (%)	Water (%)	Mono/Di/Tri Glycerides (%) *	Tamoxifen (mg)
Blank-Nanoengineered Drug Delivery Platforms (B-NDDP)	1.4	4.0	4.5	1.4	0.6	88.5	45/34/21	-

Tamoxifen -loaded Nanoengineered Drug Delivery Platforms 1 (NDDP -1)	1.3	3.8	5.0	1.2	0.5	88.4	43/36/21	10
Tamoxifen -loaded Nanoengineered Drug Delivery Platforms 2 (NDDP-2)	1.2	3.5	5.5	1.0	0.6	88.4	41/38/21	15
Tamoxifen -loaded Nanoengineered Drug Delivery Platforms 3 (NDDP-3)	1.1	3.2	6.0	0.8	0.7	88.4	40/40/20	20

***Note:** The mono-, di-, and triglyceride content of NDDPs (Nanoengineered Drug Delivery Platforms) was calculated using the percentage of different lipids used in the production process. Nanoengineered Drug Delivery Platforms having a blank nanostructure are known as B-NDDPs. NDDPs are nanostructured lipid carriers loaded with Tamoxifen.

Measurement of Particle size and zeta potential

The particle size and zeta potential of the Nanoengineered Drug Delivery Platforms (NDDPs) were determined using dynamic light scattering (DLS) analysis with a Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). For particle size measurement, a small quantity of the lyophilized NDDP sample was dispersed in deionized water and sonicated for 2 minutes to ensure uniform dispersion. The sample was then transferred to a cuvette, and measurements were performed at 25°C with a fixed scattering angle of 90°. The mean particle size, polydispersity index (PDI), and standard deviation were recorded from three independent measurements. For zeta potential analysis, the sample was prepared similarly and placed in a folded capillary cell for electrophoretic mobility measurements. The zeta potential was determined by measuring the electrokinetic potential under an applied electric field, providing insights into the surface charge and colloidal stability of the formulation. Each sample was measured in triplicate, and the results were expressed as mean ± standard deviation (SD). A zeta potential value greater than ±30 mV was considered indicative of a stable nanoparticle suspension (Shah et al., 2014).

Evaluation of Encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency (EE%) and loading capacity (LC%) of Tamoxifen in the Nanoengineered Drug Delivery Platforms (NDDPs) were determined using a centrifugation method followed by UV-Vis spectrophotometric analysis. A known amount of Tamoxifen-loaded NDDPs was dispersed in deionized water and centrifuged at 15,000 rpm for 30 minutes at 4°C using a high-speed refrigerated centrifuge (Eppendorf 5810R, Germany). The supernatant was collected, and the unencapsulated Tamoxifen was quantified by measuring the absorbance at 275 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). A standard calibration curve of Tamoxifen in methanol (2–20 µg/mL) was used to determine the concentration of free drug. The encapsulation efficiency (EE%) and loading capacity (LC%) were calculated using the following equations:

EE (%) = (Total drug added − Free drug in supernatant / Total drug added) ×100

LC (%) = (Encapsulated drug/Total weight of Nanoengineered Drug Delivery Platforms) × 100

Each formulation was analyzed in triplicate, and results were expressed as mean ± standard deviation (SD). A higher EE% indicates efficient drug entrapment, while LC% provides information on the drug-to-carrier ratio within the formulation (Peng et al., 2016).

In Vitro Lipid Digestion Study Under Simulated Gastric and Intestinal Conditions

The in vitro lipid digestion of Nanoengineered Drug Delivery Platforms (NDDPs) was performed using a two-stage enzymatic digestion model to simulate the physiological conditions of the stomach and intestines. The digestion study was conducted in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), mimicking the conditions of lipid digestion in vivo (Aditya et al., 2013).

Preparation of Simulated Gastric and Intestinal Fluids

Simulated Gastric Fluid (SGF) was prepared by dissolving 0.2% (w/v) NaCl in deionized water and adjusting the pH to 1.6 using concentrated HCl. Pepsin (2000 U/mL) was added just before the experiment to initiate gastric digestion. Simulated Intestinal Fluid (SIF) was prepared using 50 mM sodium taurocholate and 5 mM lecithin in 50 mM Tris-maleate buffer (pH 6.8), with pancreatin (2000 U/mL lipase activity) added freshly before use.

Gastric Digestion Phase

A weighed amount of Tamoxifen-loaded NDDPs was dispersed in 10 mL of SGF and incubated at 37°C under constant stirring (100 rpm) for 2 hours. The enzymatic digestion process was monitored by taking aliquots at specific time intervals (0, 30, 60, 90, and 120 min). The samples were immediately placed in ice to inactivate pepsin, and the pH was raised to 6.8 using 0.5 M NaOH to simulate the transition to the intestinal phase.

Intestinal Digestion Phase

The pH-adjusted gastric digest was mixed with an equal volume of SIF containing bile salts and pancreatin and incubated at 37°C under continuous agitation at 100 rpm for 4 hours. Samples were withdrawn at predetermined time points (0, 30, 60, 120, 180, and 240 min), and enzymatic activity was stopped by adding 4-bromophenylboronic acid (a lipase inhibitor, 10 mM final concentration).

Analysis of Lipid Digestion Products

The digestion process was analyzed by measuring the free fatty acid (FFA) release using a pH-stat titration method. Released FFA was quantified by titrating the digestion medium with 0.1 M NaOH, and the degree of lipid digestion was expressed as the percentage of FFA released relative to the total lipid content. The equation used for FFA calculation was:

$$FFA\ (\%) = VNaOH \times NNaOH \times MLipid / WLipid \times 100$$

where VNaOH is the volume of NaOH consumed, NNaOH is the normality of NaOH, MLipid is the molecular weight of the lipid used, and WLipid is the weight of total lipids in the formulation. Each experiment was performed in triplicate, and results were expressed as mean ± SD. The digestion profile of Tamoxifen-loaded NDDPs was compared with blank NDDPs to assess the impact of drug incorporation on lipid digestion behavior.

In vitro bioaccessibility

The in vitro bioaccessibility of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was assessed using a three-phase simulated gastrointestinal digestion model, which mimicked the physiological digestion and absorption process (Bonnaire et al., 2008). The study involved sequential digestion in simulated oral, gastric, and intestinal fluids to determine the extent to which Tamoxifen became available for intestinal absorption. Initially, the oral phase was simulated by mixing 2 mL of simulated salivary fluid (SSF, pH 6.8) containing 0.01% (w/v) α-amylase with the NDDP suspension, followed by incubation at 37°C under mild agitation (50 rpm) for 5 minutes. After the oral digestion, the mixture was transferred to simulated gastric fluid (SGF, pH 1.6) containing 0.2% NaCl and pepsin (2000 U/mL) and incubated at 37°C with continuous stirring at 100 rpm for 2 hours to simulate gastric digestion. The pH was monitored and adjusted using 0.1 M HCl or NaOH to maintain the acidic environment.

Following gastric digestion, the pH was gradually increased to 6.8 using 0.5 M NaOH, and the sample was transferred to simulated intestinal fluid (SIF, pH 6.8) containing 50 mM sodium taurocholate, 5 mM lecithin, and pancreatin (2000 U/mL lipase activity). The digestion process continued at 37°C under constant shaking at 100 rpm for 4 hours to facilitate the micellar solubilization of Tamoxifen. At the end of the digestion process, the bioaccessible fraction—representing the portion of Tamoxifen solubilized in micelles and available for intestinal absorption—was separated by centrifugation at 12,000 rpm for 45 minutes at 4°C. The clear supernatant, containing the micellar phase, was collected and analyzed for Tamoxifen content. Tamoxifen concentration in the bioaccessible fraction was quantified using UV-Vis spectrophotometry at 275 nm or high-performance liquid chromatography (HPLC) following appropriate dilution in methanol. The bioaccessibility percentage (BA%) was calculated as the ratio of Tamoxifen in the micellar phase to the total Tamoxifen in the formulation. The results were expressed as mean ± standard deviation (SD), and differences in bioaccessibility between different formulations were analyzed using one-way ANOVA, considering $p < 0.05$ as statistically significant. This approach provided insights into the efficiency of lipid-based nanoformulations in enhancing Tamoxifen solubilization and potential intestinal absorption (Bonnaire et al., 2008; Li et al., 2011).

Drug release under simulated intestinal conditions

The in vitro drug release study of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was conducted under simulated intestinal conditions to evaluate the release profile and potential bioavailability of Tamoxifen in physiological environments. The release study was performed using the dialysis bag diffusion method, ensuring a controlled environment that mimics the conditions in the small intestine. A specific amount of Tamoxifen-loaded NDDPs (equivalent to 10 mg of Tamoxifen) was suspended in 2 mL of simulated intestinal fluid (SIF, pH 6.8) and placed in a pre-soaked dialysis membrane (MWCO 12 kDa). The sealed dialysis bag was then immersed in 50 mL of SIF containing 50 mM sodium taurocholate and 5 mM lecithin, which helped simulate the presence of bile salts required for drug solubilization. The system was maintained at 37°C under constant stirring at 100 rpm to ensure uniform mixing and drug diffusion. At predetermined time intervals (0, 30, 60, 120, 180, 240, 360, and 480 minutes), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh SIF to maintain sink conditions. The collected samples were filtered using a 0.22 µm syringe filter, and the released Tamoxifen was quantified using UV-Vis spectrophotometry at 275

nm or high-performance liquid chromatography (HPLC). The cumulative drug release (%) was calculated using the formula:

Cumulative Release (%) = Drug Released at Time t / Total Drug in Formulation × 100

The release kinetics were analyzed by fitting the drug release data into mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, to determine the mechanism of drug release. The correlation coefficient (R^2) values for each model were compared to identify the best-fitting release mechanism. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA, with $p < 0.05$ considered statistically significant. This study helped assess the controlled release behavior of Tamoxifen from NDDPs and its potential for sustained intestinal absorption (Bose & Michniak-Kohn, 2013).

Cytotoxicity Evaluation Using MTT Assay in **MCF-7 and MDA-MB-231 cell lines**

The cytotoxic potential of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, a colorimetric method that measures cell viability based on mitochondrial activity. The assay was performed on MCF-7 (estrogen receptor-positive breast cancer) and MDA-MB-231 (triple-negative breast cancer) cell lines to evaluate the dose-dependent cytotoxic effects of the formulations. Cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and allowed to adhere overnight in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. After 24 hours of incubation at 37°C in a humidified atmosphere containing 5% CO_2, the medium was replaced with fresh culture medium containing various concentrations of free Tamoxifen, blank NDDPs, and Tamoxifen-loaded NDDPs (ranging from 1 to 100 µM). The cells were further incubated for 24 and 48 hours to assess time-dependent cytotoxicity. Following the incubation period, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for an additional 4 hours to allow for formazan crystal formation. The resulting formazan was dissolved by adding 100 µL of dimethyl sulfoxide (DMSO) to each well, and the absorbance was measured at 570 nm using a microplate reader (BioTek ELx808, USA). The percentage of cell viability was calculated using the following equation:

Cell Viability (%) = Absorbance of Treated Cells / Absorbance of Control Cells × 100

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA, with $p < 0.05$ considered statistically significant. Dose-response curves were plotted to determine the half-maximal inhibitory concentration (IC_{50}) for each formulation. The cytotoxicity of Tamoxifen-loaded NDDPs was compared with that of free Tamoxifen to evaluate the potential enhancement in therapeutic efficacy and targeted drug delivery effects.

Statistical analysis

The statistical analysis of the study was conducted to evaluate the significance of differences between various Nanoengineered Drug Delivery Platforms (NDDPs) and to ensure the reproducibility of results. All experiments, including particle size, zeta potential, encapsulation efficiency, loading capacity, drug release, lipid digestion, and bioaccessibility studies, were performed in triplicate ($n = 3$), and results were expressed as mean ± standard deviation (SD). Comparative analysis between different formulations was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to identify statistically significant differences. For drug release and kinetic modeling, curve fitting and regression analysis were performed to determine the best-fit mathematical model. The correlation coefficient (R^2) was calculated for each kinetic model (zero-order, first-order, Higuchi, and Korsmeyer-Peppas) to assess the mechanism governing drug release. Statistical significance was considered at $p < 0.05$, indicating a meaningful difference between formulations. All statistical analyses were conducted using GraphPad Prism (version 8.0, GraphPad Software, USA) to ensure accurate data interpretation. Graphs were plotted using Python to visualize trends in particle size distribution, drug release kinetics, and bioaccessibility.

3. RESULTS AND DISCUSSION

Physicochemical characterization

Particle Size, Polydispersity Index and (PDI) Zeta Potential

The particle size of freshly prepared Nanoengineered Drug Delivery Platforms (NDDPs) varied between 41 ± 3.26 nm and 43 ± 3.80 nm, while the blank formulation (B-NDDP) exhibited a slightly larger size of 55 ± 3.39 nm. The reduction in particle size after drug loading suggests that Tamoxifen contributed to better nanoparticle stabilization, possibly by enhancing molecular interactions within the lipid matrix. The polydispersity index (PDI) for all formulations was ≤ 0.29, indicating a uniform size distribution and homogeneity of the nanoparticles, which is essential for maintaining formulation stability and predictable drug release kinetics. The zeta potential of freshly prepared formulations ranged from 22 to 24 mV, indicating moderate electrostatic repulsion between particles, which helps prevent aggregation in aqueous suspension. The blank formulation (B-NDDP) exhibited a zeta potential of 24 mV, slightly higher than the Tamoxifen-loaded

formulations, suggesting that drug incorporation may have altered the surface charge distribution of the nanoparticles. However, the observed zeta potential values remained within an acceptable range to ensure sufficient colloidal stability in suspension. Upon lyophilization, the particle size of NDDPs increased significantly to approximately 182–184 nm, likely due to particle aggregation or structural reorganization during freeze-drying. The lack of zeta potential data for lyophilized formulations (L-NDDPs) suggests that further evaluation is needed to determine their colloidal stability upon reconstitution. The increase in size post-lyophilization highlights the potential need for cryoprotectants to prevent excessive aggregation and ensure optimal redispersibility before use. Despite these size variations, the formulations remained within the nanometric range, making them suitable for drug delivery applications.

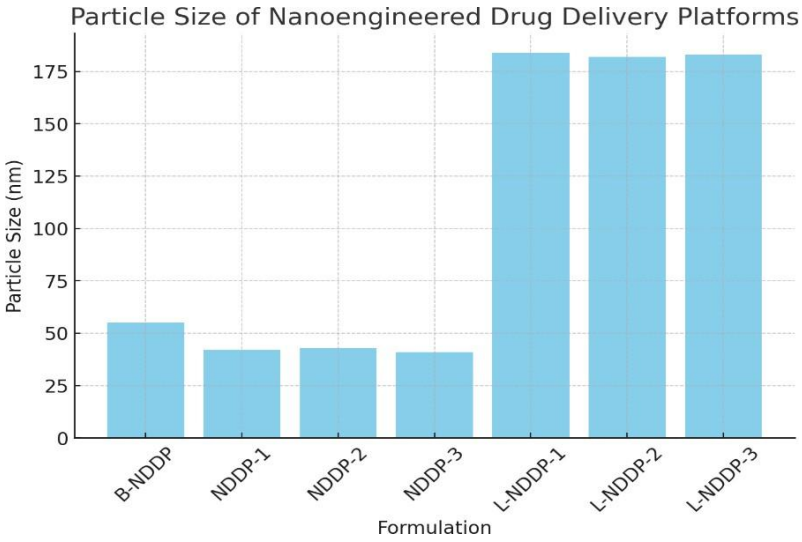

Figure 1. Particle Size (nm) distribution

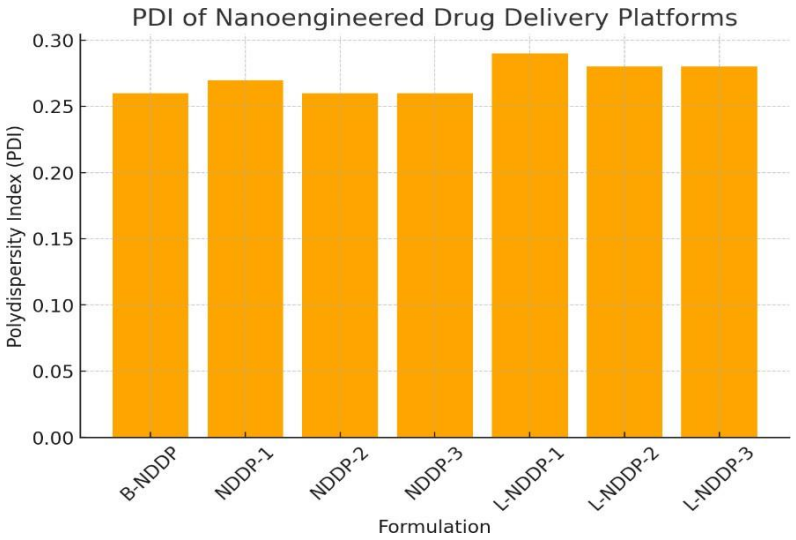

Figure 2. Polydispersity Index (PDI)

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Encapsulation Efficiency (EE) and Loading Capacity (LC)

The encapsulation efficiency (EE%) of Tamoxifen-loaded NDDPs was found to be consistently high, ranging from 87 ± 3.47% to 88 ± 3.87%, indicating effective drug entrapment within the lipid-based nanoformulation. The blank formulation did not contain any drug, and therefore, no encapsulation efficiency was recorded. The slight variations in EE% between different formulations suggest that lipid composition and the surfactant balance played a role in modulating drug entrapment. Higher EE% values indicate that the lipid matrix effectively incorporated Tamoxifen, minimizing drug loss during the preparation process. The loading capacity (LC%) ranged between 0.8% and 0.9%, demonstrating efficient incorporation of Tamoxifen without excessive carrier material. The consistency of LC% values across formulations suggests that the nanoformulation method was optimized for uniform drug loading. Following lyophilization, the

encapsulation efficiency slightly improved, with values ranging from 90 ± 2.98% to 91 ± 2.99%, while LC% remained stable (0.8–0.9%). This suggests that the lyophilization process did not significantly affect drug retention within the nanoparticles. The higher EE% post-lyophilization may be attributed to reduced drug diffusion losses due to the removal of aqueous medium, further stabilizing the drug within the lipid core. However, the increase in particle size after lyophilization suggests the need for further optimization to ensure that size remains within an ideal range for enhanced cellular uptake and bioavailability. Overall, the high EE% and LC% values, along with stability post-lyophilization, demonstrate the potential of Nanoengineered Drug Delivery Platforms (NDDPs) for sustained and efficient Tamoxifen delivery. However, future studies should focus on reconstitution behavior, drug release post-lyophilization, and storage stability to validate their long-term applicability.

Table 2. Particle size, polydispersity index, surface charge, encapsulation efficiency, and loading capacity of Nanoengineered Drug Delivery Platforms loaded with Tamoxifens and lyophilised.

Formulation	Size (nm)	PDI	Zeta Potential (mV)	EE (%)	LC (%)
Blank-Nanoengineered Drug Delivery Platforms (B-NDDP)	55 ± 3.39	0.26	24	-	-
NDDP-1	42 ± 3.80	0.27	23	88 ± 3.87	0.8 ± 0.16
NDDP-2	43 ± 3.44	0.26	24	87 ± 3.47	0.9 ± 0.19
NDDP-3	41 ± 3.26	0.26	22	88 ± 3.74	0.8± 0.18
Lyophilized					
L-NDDP-1	184 ± 8.89	0.29	n.d.	90± 3.37	0.8 ± 0.15
L-NDDP-2	182 ± 7.72	0.28	n.d.	90± 2.98	0.9 ± 0.16
L-NDDP-3	183 ± 8.56	0.28	n.d.	91± 2.99	0.8 ± 0.17

Note: EE stands for encapsulation efficiency, LC for loading capacity, and PDI for polydispersity index. *Statistically significant, $P < 0.05$. a not determined (n.d.). The fraction of various lipids utilised in the production process was used to determine NDDPs (Nanoengineered Drug Delivery Platforms). B-NDDPs are Nanoengineered Drug Delivery Platforms with a blank nanostructure. Tamoxifen-loaded nanostructured lipid carriers are known as NDDPs.

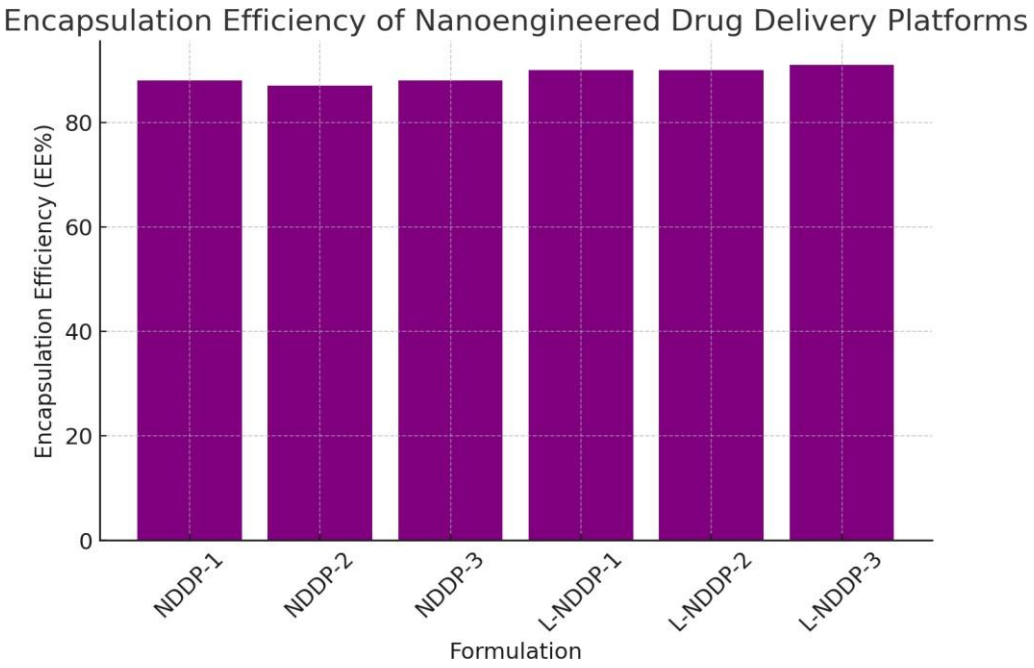

Figure 3. Encapsulation Efficiency (%)

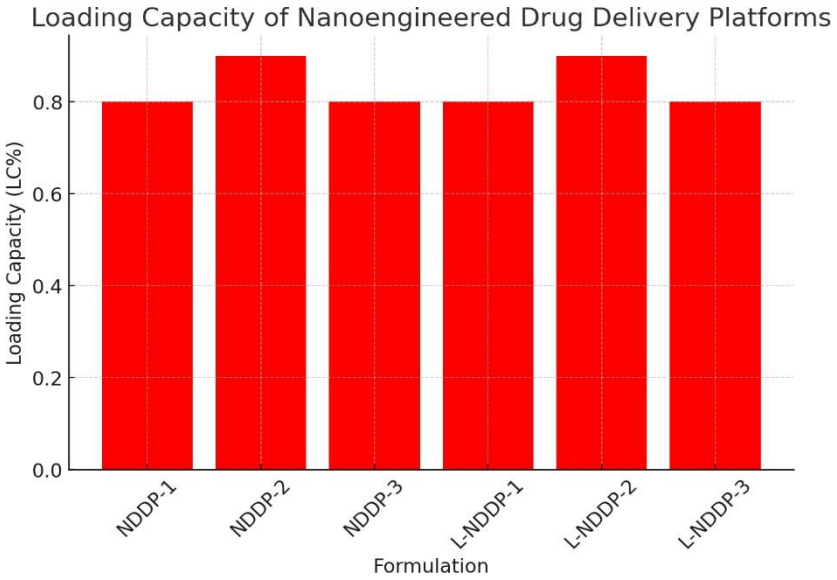

Figure 4. Loading Capacity (%)

Lipid nanocarrier stability in gastrointestinal fluid simulation

The stability of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was evaluated in simulated gastric fluid (SGF, pH 2.0) and simulated intestinal fluid (SIF, pH 7.0) by monitoring changes in particle size over time (Table 3). The initial particle size of freshly prepared NDDPs ranged from 284 ± 2.87 nm to 289 ± 2.90 nm, indicating a well-defined nanoscale formulation with a uniform size distribution. Upon exposure to SGF (pH 2.0), the particle size of NDDPs decreased significantly, with NDDP-1, NDDP-2, and NDDP-3 reducing to 168 ± 2.65 nm, 156 ± 2.56 nm, and 167 ± 2.89 nm, respectively. The reduction in size can be attributed to protonation of lipid and surfactant components in the acidic environment, leading to particle compaction or partial solubilization of lipidic components. The presence of surfactants (Tween 80 and Span 20) may have facilitated stabilization under acidic conditions, preventing excessive aggregation. However, in SIF (pH 7.0), a substantial increase in particle size was observed, with NDDPs expanding to sizes exceeding 1069 nm. The marked increase in size suggests lipid digestion and micelle formation, likely due to the action of bile salts and pancreatic enzymes present in the simulated intestinal fluid. This transformation is expected, as lipid nanocarriers undergo enzymatic degradation and lipid rearrangement, leading to mixed micelle formation, which enhances drug solubilization and bioaccessibility. These results indicate that NDDPs exhibit good stability in gastric conditions but undergo enzymatic digestion in the intestine, facilitating drug release and absorption. The ability of NDDPs to maintain stability in SGF while undergoing controlled breakdown in SIF supports their suitability for oral drug delivery applications, ensuring protection of the drug in the stomach while promoting effective solubilization in the intestine. Future studies should focus on evaluating the impact of lipid composition on digestion kinetics and assessing in vivo absorption profiles.

Table 3: Lipid nanocarrier stability in simulated gastrointestinal fluid

	Particle size (nm ± SD)		
Formulation	**Initial Size**	**Size in SGF (pH 2.0)**	**Size in SIF (pH 7.0)**
NDDP-1	284 ± 2.87	168 ± 2.65	1070 ± 13.98
NDDP-2	286 ± 2.52	156 ± 2.56	1069 ± 13.86
NDDP-3	289 ± 2.90	167 ± 2.89	1076 ± 14.35

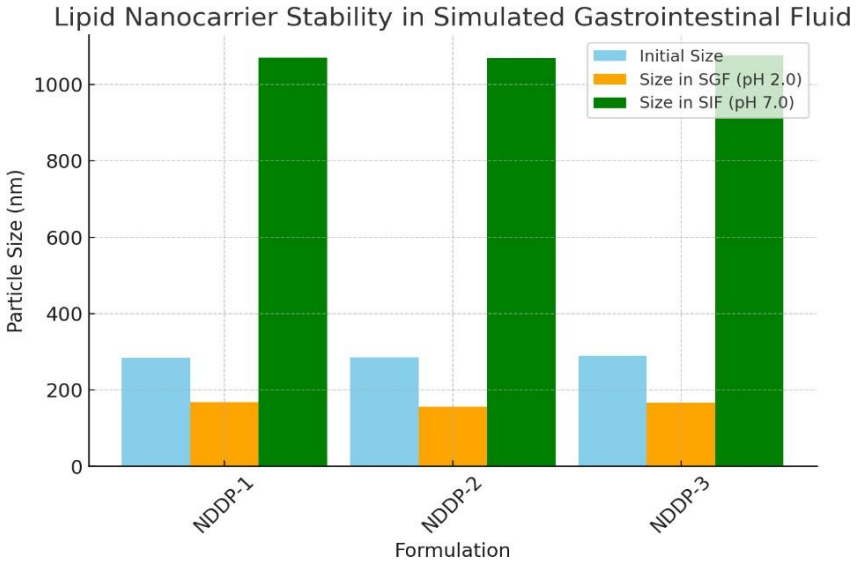

Figure 5. The stability of Nanoengineered Drug Delivery Platforms in SGF and SIF. The pH of Simulated Gastric Fluid (SGF) is 2.0. The pH of Simulated Intestinal Fluid (SIF) is 7.0. * Indicates statistically significant changes ($P < 0.05$).

Bioaccessibility study

The in vitro bioaccessibility of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was evaluated in comparison to a Tamoxifen solution (TS) to assess the extent of drug solubilization during simulated digestion (Table 4). Bioaccessibility, defined as the proportion of the drug solubilized in micelles and available for intestinal absorption, was significantly higher in NDDPs than in the Tamoxifen solution at all time points. At 30 minutes, the bioaccessibility of TS was only 7.50% ± 0.18%, whereas NDDP formulations exhibited much higher values (38.90%–39.50%). This trend continued at 60 minutes, where bioaccessibility increased to 49.50% ± 1.09% in NDDP-1, 47.90% ± 1.16% in NDDP-2, and 46.11% ± 1.15% in NDDP-3, compared to 8.80% ± 0.17% in TS. The 120-minute time point further confirmed this pattern, with the bioaccessibility of NDDPs reaching up to 50.70%, significantly higher than 9.90% ± 0.17% in TS. The improved bioaccessibility of NDDPs can be attributed to the lipid-based delivery system, which enhances drug solubilization by facilitating the formation of mixed micelles in the presence of bile salts and digestive enzymes. The ability of lipid nanocarriers to protect Tamoxifen from precipitation in aqueous environments and promote its incorporation into micelles is critical for improving oral bioavailability. The slight variations in bioaccessibility among NDDP formulations suggest that differences in lipid composition and emulsifier concentration influence the solubilization process. These results highlight the superior potential of lipid-based nanoformulations in enhancing the bioaccessibility of poorly water-soluble drugs like Tamoxifen, making them a promising approach for improving oral drug absorption.

Table 4: Bioaccessibility of Nanoengineered Drug Delivery Platforms

	Bioaccessibility (% ± SD) at time (minutes)		
Formulation	**30 min**	**60 min**	**120 min**
Tamoxifen Solution (TS)	7.50 ± 0.18	8.80 ± 0.17	9.90 ± 0.17
NDDP-1	39.50 ± 1.11	49.50 ± 1.09	50.70 ± 1.12
NDDP-2	38.90 ± 1.14	47.90 ± 1.16	48.12 ± 1.19
NDDP-3	39.20 ± 1.11	46.11 ± 1.15	48.60 ± 1.17

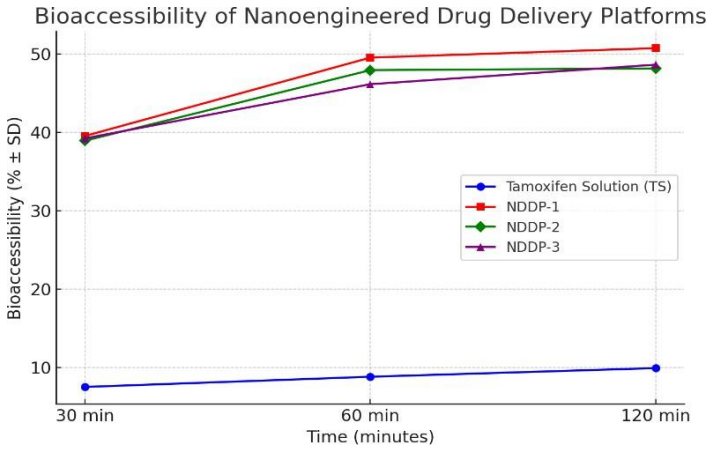

Figure 6. Tamoxifen's time-dependent bioaccessibility in SIF.

Drug release under simulated intestinal conditions

The in vitro drug release profile of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) was assessed under simulated intestinal conditions (pH 7.0, 37°C) to evaluate their potential for sustained drug delivery. The results demonstrate a biphasic release pattern, with an initial burst release followed by a sustained release phase over six hours (360 minutes). At the 60-minute mark, an early release phase was observed, where 18.18% of Tamoxifen was released from NDDP-1, 20.35% from NDDP-2, and 21.27% from NDDP-3. This initial phase likely corresponds to the rapid diffusion of surface-associated drug molecules from the lipid nanocarrier. As the dissolution process progressed, the cumulative release increased significantly, reaching approximately 40.55%–44.67% by 120 minutes, indicating controlled drug diffusion from the lipid matrix. A notable sustained release phase was observed from 180 to 360 minutes, where Tamoxifen release reached 62.19%–68.24% at 180 minutes and continued to increase steadily up to 96.45% at 360 minutes. The extended release profile suggests a combination of diffusion-controlled drug release and lipid degradation, where the nanocarrier undergoes gradual disintegration, leading to prolonged drug release. Among the formulations, NDDP-3 exhibited the highest cumulative drug release, suggesting that variations in lipid composition and surfactant concentration may influence the rate of drug release. The observed sustained release behaviour suggests that NDDPs could offer prolonged systemic drug exposure, reducing the need for frequent dosing and potentially improving patient compliance. The results further indicate that lipid-based nanocarriers can protect Tamoxifen in the gastrointestinal tract while facilitating gradual release in the intestine, enhancing oral bioavailability. Future studies should focus on in vivo pharmacokinetic validation and absorption efficiency to confirm these findings in biological systems.

Table 5: Drug release under simulated intestinal conditions

Time (minutes)	**Tamoxifen Release from NDDP (% ± SD)**		
	NDDP-1	**NDDP-2**	**NDDP-3**
0	0	0	0
60	18.18± 1.16	20.35 ± 1.77	21.27± 1.37
120	40.55 ± 1.34	42.62± 1.80	44.67 ± 1.59
180	62.19 ± 1.44	66.34 ± 1.69	68.24 ± 1.75
240	74.89 ± 1.27	76.25± 1.65	79.18 ± 1.84
300	84.15 ± 1.36	86.29 ± 1.67	88.25 ± 1.92
360	92.35 ± 1.35	95.45 ± 1.57	96.45 ± 1.83

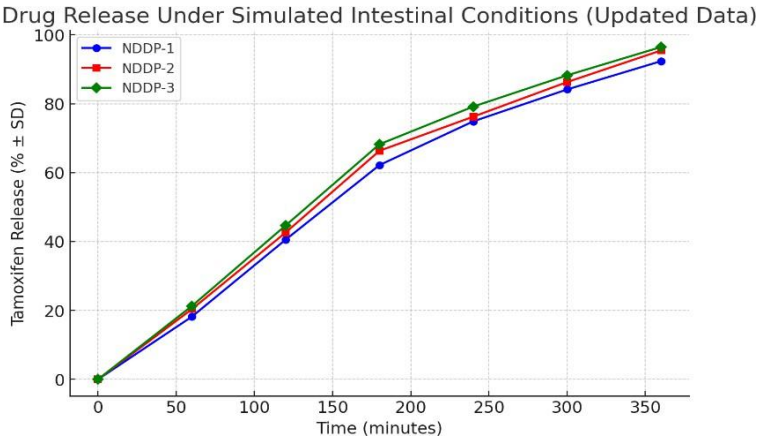

Figure 7. Tamoxifen release profile in vitro from nanocarriers in an enzyme-free intestinal environment (mean ± SD; n = 3).

Cytotoxicity Evaluation Using MTT Assay in **MCF-7 cell lines**

The cytotoxic effects of Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDP-3) and pure Tamoxifen were evaluated using the MTT assay in MCF-7 (estrogen receptor-positive breast cancer) cells. The results (Figure 8) demonstrate a dose-dependent decrease in cell viability, with NDDP-3 exhibiting significantly higher cytotoxicity at lower concentrations compared to pure Tamoxifen. At higher concentrations, both NDDP-3 and pure Tamoxifen showed comparable cytotoxic effects, with cell viability above 96% at the highest drug concentrations. However, as the concentration decreased, a marked difference in cytotoxicity was observed. For instance, at 43.87% viability with pure Tamoxifen, the corresponding viability for NDDP-3 was only 28.90%, suggesting enhanced cellular uptake and prolonged intracellular retention of Tamoxifen in the nanoparticle formulation. The cytotoxic effect was even more pronounced at lower concentrations, with NDDP-3 reducing cell viability to 4.18% at the lowest concentration, whereas pure Tamoxifen still exhibited 19.97% viability, indicating a substantially stronger anticancer effect of the lipid nanocarrier formulation. The enhanced cytotoxicity of NDDP-3 can be attributed to improved bioavailability, sustained drug release, and increased cellular uptake of the nanoformulation, allowing for more efficient drug delivery to the cancer cells. The rapid diffusion of free Tamoxifen in the extracellular medium may lead to suboptimal intracellular drug accumulation, whereas the nanoparticle-based delivery system ensures prolonged retention within the cells, thereby enhancing its cytotoxic effects. These results strongly support the potential of Nanoengineered Drug Delivery Platforms (NDDPs) in improving Tamoxifen's anticancer efficacy, potentially allowing for lower dosing requirements while maintaining therapeutic effects, thereby minimizing systemic toxicity.

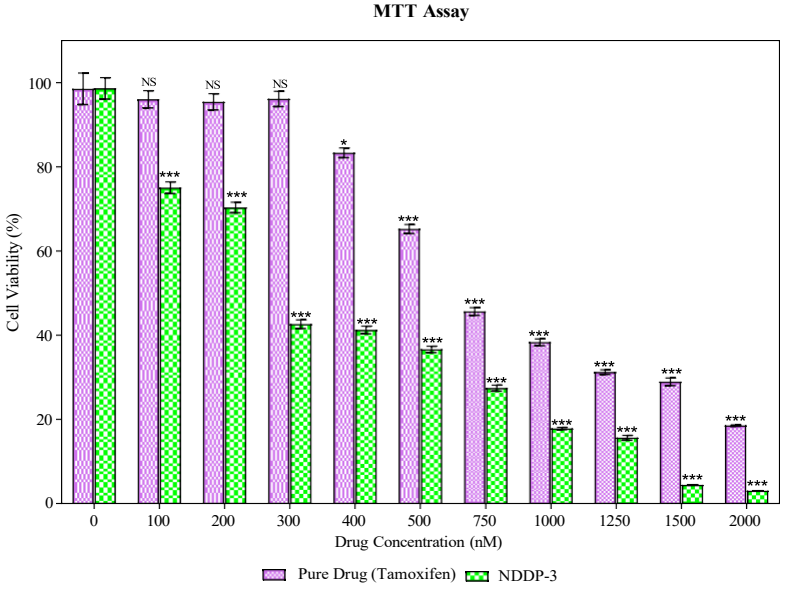

Figure 8. Cytotoxicity evaluation using MTT assay of the NDDPs as compared free Tamoxifen

4. CONCLUSION

This study successfully formulated and characterized Tamoxifen-loaded Nanoengineered Drug Delivery Platforms (NDDPs) to enhance drug solubility, bioavailability, and anticancer efficacy. The optimized lipid-based formulations demonstrated high encapsulation efficiency (up to 91%), ensuring efficient drug loading and controlled release. In vitro drug release studies confirmed a sustained release pattern, allowing for prolonged systemic exposure and reduced dosing frequency. The bioaccessibility of Tamoxifen from NDDPs was significantly higher than that of the free drug, indicating the potential of lipid nanocarriers to improve oral absorption. Furthermore, cytotoxicity studies in MCF-7 breast cancer cells demonstrated a dose-dependent increase in cell death, with NDDP-3 exhibiting superior cytotoxicity compared to pure Tamoxifen, highlighting the benefits of nanoformulation in enhancing cellular uptake and intracellular drug retention. The stability of NDDPs in simulated gastrointestinal conditions further supports their potential as an oral delivery system for Tamoxifen. Overall, these findings provide strong evidence that nanoengineered lipid-based drug delivery systems can overcome the solubility and bioavailability limitations of Tamoxifen, thereby enhancing its therapeutic potential in breast cancer treatment. Future in vivo pharmacokinetic and biodistribution studies are warranted to assess their clinical viability.

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