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"FORMULATION OF PACLITAXEL-ENCAPSULATED POLYMERIC MICELLES FOR TARGETED BREAST CANCER THERAPY"

Sachin Pachani[1*], Tapaskumar M. Shah[2]

[1*]Atmiya Institute of Pharmacy (Atmiya university- Rajkot- 360005)
[2]Team Leader Alembic pharmaceutical Ltd Vadodara-390003

***Corresponding Authors:** Sachin Pachani
*Atmiya Institute of Pharmacy (Atmiya university- Rajkot- 360005),
sachinpachani2002@gmail.com

Abstract
Breast cancer remains a leading cause of cancer-related mortality among women worldwide, with therapeutic outcomes often limited by the poor solubility, systemic toxicity, and multidrug resistance associated with conventional paclitaxel formulations. In the present study, paclitaxel-loaded polymeric micelles were successfully developed using amphiphilic block copolymers through a solvent evaporation–thin film hydration technique. The optimized micelles exhibited a nanoscale size (~100 nm), narrow polydispersity, negative zeta potential, and spherical morphology as confirmed by dynamic light scattering and transmission electron microscopy. High encapsulation efficiency (~90%) and satisfactory drug loading (~8%) demonstrated the ability of the micelles to solubilize paclitaxel efficiently. In vitro release studies revealed a biphasic, sustained release pattern, with accelerated drug release under acidic conditions mimicking the tumor microenvironment. Cytotoxicity assays showed significantly enhanced anticancer activity of micellar paclitaxel compared with free paclitaxel in MCF-7 and MDA-MB-231 cell lines, with nearly twofold lower IC_{50} values. Confocal microscopy and flow cytometry confirmed superior cellular uptake of micelles, correlating with enhanced apoptotic activity. These findings indicated that polymeric micelles provided an effective strategy for improving solubility, stability, tumor targeting, and therapeutic efficacy of paclitaxel. This nanocarrier system holds strong potential for further preclinical development and clinical translation in breast cancer therapy.

Keywords: Paclitaxel, Polymeric micelles, Breast cancer, Targeted drug delivery, Nanocarrier systems, Cytotoxicity, Sustained release

1. Introduction

1.1 Global Burden of Breast Cancer

Breast cancer is the most frequently diagnosed malignancy among women globally and remains a leading cause of cancer-related mortality. According to the Global Cancer Observatory (GLOBOCAN) 2018, it accounted for over 2.08 million new cases and approximately 626,000 deaths worldwide, representing about 24% of all female cancers [1]. In both developed and developing countries, its incidence is rising due to factors such as urbanization, lifestyle changes, delayed childbirth, reduced breastfeeding, and increased prevalence of obesity and sedentary behavior [2].

In India, breast cancer has surpassed cervical cancer to become the most common cancer among women, with an estimated age-standardized incidence rate of 25.8 per 100,000 and mortality rate of 12.7 per 100,000 [3]. Despite advances in diagnostic techniques and systemic therapies, survival outcomes vary drastically between high-income and low-to-middle-income countries. In high-income countries, the five-year survival rate exceeds 85%, while in resource-limited settings, it often falls below 60% due to delayed diagnosis and limited access to effective treatments [4].
This rising burden highlights the pressing need for more effective, affordable, and safer therapeutic interventions to manage breast cancer, particularly in advanced and metastatic stages where treatment outcomes remain unsatisfactory [5].

1.2 Limitations of Conventional Paclitaxel Therapy

Paclitaxel, a diterpenoid isolated from the bark of *Taxus brevifolia*, is a first-line chemotherapeutic agent used in breast, ovarian, and lung cancers. It exerts its anticancer activity by binding to the β-tubulin subunit of microtubules, thereby promoting tubulin polymerization and stabilizing microtubules against depolymerization. This stabilization disrupts mitotic spindle formation, leading to cell cycle arrest and apoptosis [6]. Despite its remarkable efficacy, conventional paclitaxel therapy suffers from significant drawbacks that restrict its clinical potential.

Poor aqueous solubility

Paclitaxel is highly lipophilic, with an aqueous solubility of less than 0.3 μg/mL, making it extremely difficult to administer in physiological fluids [7]. To overcome this, commercial formulations such as Taxol® employ a solvent system of Cremophor EL (polyethoxylated castor oil) and ethanol, which solubilizes the drug but introduces severe hypersensitivity reactions, hemolysis, and neurotoxicity [8]. Even with premedication using corticosteroids and antihistamines, many patients experience adverse infusion-related reactions [9].

Systemic toxicity and narrow therapeutic window

The solvent system not only contributes to acute hypersensitivity but also exacerbates systemic toxicity, including peripheral neuropathy, myelosuppression, and hepatotoxicity [10]. These dose-limiting toxicities often necessitate treatment delays or dose reductions, thereby reducing therapeutic effectiveness. Moreover, paclitaxel exhibits non-specific distribution in the body, leading to damage in normal tissues alongside tumor cells [11].

Multidrug resistance (MDR)

Another major limitation is the development of multidrug resistance in tumor cells. Paclitaxel is a known substrate for P-glycoprotein (P-gp), a drug efflux pump that actively removes chemotherapeutic agents from cancer cells, reducing intracellular drug concentration and leading to therapeutic failure [12]. MDR is a significant challenge in metastatic breast cancer, where repeated exposure to paclitaxel results in cross-resistance to other chemotherapeutic agents, severely limiting treatment options [13].

Pharmacokinetic variability

Paclitaxel exhibits nonlinear pharmacokinetics, with interpatient variability due to differences in hepatic metabolism (CYP2C8 and CYP3A4 pathways) and plasma protein binding [14]. Such variability complicates dose optimization and contributes to unpredictable therapeutic outcomes.
Given these limitations, there has been considerable interest in developing novel drug delivery systems such as liposomes, nanoparticles, albumin-bound formulations, and polymeric micelles to improve the solubility, stability, and tumor-specific delivery of paclitaxel [15]. Among these, polymeric micelles have emerged as particularly promising candidates due to their ability to enhance aqueous solubility, prolong circulation time, exploit the enhanced permeability and retention (EPR) effect, and potentially overcome MDR mechanisms [16].

1.3 Nanotechnology as a Solution in Cancer Therapy

In recent decades, nanotechnology has emerged as a transformative approach in oncology, offering novel strategies to overcome the inherent limitations of conventional chemotherapeutics such as paclitaxel. Nanoscale drug delivery systems—including micelles, liposomes, dendrimers, polymeric nanoparticles, and solid lipid nanoparticles—have been extensively explored to enhance solubility, improve pharmacokinetics, and achieve tumor-specific drug accumulation [17]. These nanocarriers exploit the enhanced permeability and retention (EPR) effect, a phenomenon arising from the leaky vasculature and impaired lymphatic drainage of solid tumors, which allows nanoparticles in the size range of 10–200 nm to preferentially accumulate at tumor sites [18].

Liposomes were among the first nanocarriers approved for clinical use, exemplified by Doxil® (liposomal doxorubicin), which demonstrated reduced cardiotoxicity compared to free doxorubicin [19]. Similarly, albumin-bound nanoparticles such as Abraxane® (nab-paclitaxel) provided an alternative to Cremophor EL-based paclitaxel formulations, reducing solvent-associated hypersensitivity reactions and improving therapeutic outcomes [20]. Despite these advances, limitations such as premature drug release, instability during storage, and relatively low drug loading capacity restrict the broader application of some nanocarrier systems [21].

Against this backdrop, polymeric micelles have gained increasing attention due to their simple preparation, high stability, superior drug loading, and ability to solubilize hydrophobic drugs like paclitaxel while maintaining nanoscale dimensions that facilitate tumor targeting [22].

1.4 Polymeric Micelles as Promising Carriers

Polymeric micelles are self-assembled colloidal structures formed by amphiphilic block copolymers in aqueous solution, typically characterized by a hydrophobic core and a hydrophilic corona [23]. The hydrophobic core serves as a reservoir for poorly water-soluble drugs such as paclitaxel, while the hydrophilic shell (often polyethylene glycol, PEG) provides steric stabilization, prolongs systemic circulation, and reduces uptake by the reticuloendothelial system (RES) [24].

The unique architecture of polymeric micelles offers several key advantages:

1. Improved solubility – Hydrophobic drugs are efficiently encapsulated in the micelle core, significantly enhancing their aqueous solubility [25].

2. Tumor targeting via the EPR effect – Nanometer size enables passive accumulation at tumor sites, thereby increasing local drug concentration while reducing off-target toxicity [26].

3. Controlled and sustained release – Drugs can be released in a controlled manner, maintaining therapeutic concentrations over extended periods and reducing the frequency of dosing [27].

4. Reduced systemic toxicity – By limiting non-specific distribution, polymeric micelles minimize adverse effects such as hypersensitivity, myelosuppression, and neuropathy often observed with free paclitaxel [28].

5. Potential to overcome multidrug resistance (MDR) – Micellar encapsulation may reduce P-glycoprotein mediated efflux, thereby improving intracellular drug retention and efficacy against resistant cancer cells [29].

Clinical studies further support the translational potential of micellar systems. For instance, Genexol-PM®, a polymeric micelle formulation of paclitaxel using PEG–poly(D,L-lactic acid) (PEG–PLA), demonstrated enhanced antitumor activity and a favorable safety profile in breast cancer patients compared to conventional Taxol® [30]. These results underscore the promise of polymeric micelles as next-generation carriers for hydrophobic anticancer drugs.

1.5 Research Gap and Novelty of the Present Study

Although several nanocarrier systems for paclitaxel delivery have been developed, there remain significant challenges in optimizing stability, drug loading efficiency, controlled release, and tumor-specific accumulation. Existing commercial formulations such as Abraxane® and Genexol-PM® have improved safety compared to solvent-based paclitaxel; however, their clinical benefit remains

limited by issues including variable pharmacokinetics, suboptimal biodistribution, and incomplete mitigation of systemic toxicities [31]. Furthermore, many formulations fail to adequately address the problem of MDR, which remains a leading cause of treatment failure in advanced breast cancer [32].

Additionally, while liposomes and albumin-bound nanoparticles have reached the market, polymeric micelles represent a relatively underexplored platform, with fewer FDA-approved products despite their superior physicochemical properties. There is a pressing need for systematic research on the design, formulation, and evaluation of paclitaxel-encapsulated polymeric micelles, particularly focusing on their in vitro cytotoxicity, in vivo antitumor efficacy, and ability to reduce toxicity compared to conventional formulations [33].

The novelty of the present study lies in the development of a paclitaxel-encapsulated polymeric micellar system specifically optimized for targeted breast cancer therapy. By carefully selecting biocompatible polymers, optimizing formulation parameters, and conducting detailed physicochemical and biological evaluations, this study aims to contribute to the advancement of micellar nanocarriers as clinically translatable drug delivery platforms for breast cancer management.

1.6 Aim and Objectives of the Present Work

The overarching aim of this research was to **develop and evaluate paclitaxel-loaded polymeric micelles for targeted breast cancer therapy**. The specific objectives are:

1. **Formulation development**: To prepare paclitaxel-encapsulated polymeric micelles using amphiphilic block copolymers through optimized fabrication techniques.
2. **Physicochemical characterization**: To determine particle size, polydispersity index (PDI), zeta potential, morphology, drug loading, and encapsulation efficiency of the micelles.
3. **In vitro evaluation**: To assess the drug release profile, cytotoxicity against breast cancer cell lines, cellular uptake, and apoptosis induction potential of the micelles.
4. **Comparative analysis**: To compare the performance of micellar paclitaxel with conventional paclitaxel formulations in terms of efficacy and toxicity.

2. Materials and Methods

2.1 Materials

Drug and polymers

Paclitaxel (PTX, purity ≥99%) was obtained from a certified pharmaceutical supplier. Amphiphilic biodegradable polymers, including **poly(ethylene glycol)-block-poly(lactic acid) (PEG–PLA)** and **poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG–PCL)**, were used as micelle-forming agents. PEG provided a hydrophilic corona to enhance systemic circulation time, while PLA or PCL constituted the hydrophobic core for efficient encapsulation of paclitaxel. These polymers were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Solvents and reagents

Analytical and HPLC-grade solvents, including acetone, ethanol, methanol, dichloromethane (DCM), and dimethyl sulfoxide (DMSO), were used for micelle preparation and chromatographic analysis. Phosphate-buffered saline (PBS, pH 7.4), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and penicillin-streptomycin solution were procured from HiMedia (Mumbai, India) and Gibco (Thermo Fisher Scientific, USA). All reagents were of analytical grade, and deionized water was prepared using a Milli-Q purification system.

Cell lines

Human breast cancer cell lines **MCF-7 (estrogen receptor-positive)** and **MDA-MB-231 (triple-negative)** were used to evaluate the cytotoxic efficacy of paclitaxel-loaded polymeric micelles. These cell lines represented distinct breast cancer subtypes and allowed assessment of formulation performance across hormone-dependent and aggressive metastatic models. The cells were obtained

from the National Centre for Cell Science (NCCS), Pune, India, and were cultured according to standard protocols.

Reference drug and controls

Commercial **Taxol® (Cremophor EL-based paclitaxel injection)** was used as a reference control for comparison with the polymeric micelle formulation. Blank micelles (without drug) were prepared and used as additional controls in in vitro and in vivo studies.

2.2 Preparation of Polymeric Micelles

Paclitaxel-loaded polymeric micelles were prepared using a modified **solvent evaporation and thin-film hydration method**. Briefly, accurately weighed amounts of paclitaxel and amphiphilic polymers (**PEG–PLA** or **PEG–PCL**) were dissolved in a mixture of dichloromethane (DCM) and methanol (3:1, v/v) to obtain a clear organic solution. The solvent mixture was evaporated under reduced pressure using a rotary evaporator at 40 °C until a thin, uniform polymer–drug film was formed along the inner wall of a round-bottom flask. The dried film was kept under vacuum overnight to ensure complete removal of residual solvents.

The thin film was then hydrated with phosphate-buffered saline (PBS, pH 7.4) under gentle stirring at 200 rpm for 30 minutes at room temperature, allowing the spontaneous self-assembly of micelles. The resulting suspension was further sonicated using a probe sonicator (30% amplitude, 5 cycles of 30 seconds with 30-second intervals) to reduce particle size and ensure uniform micelle distribution.

To remove free drug and residual organic solvents, the micellar dispersion was subjected to **dialysis** against distilled water for 24 hours using a dialysis membrane (molecular weight cut-off: 12–14 kDa), with water being replaced every 4 hours. The final formulation was filtered through a 0.22 µm sterile filter to ensure sterility and to eliminate aggregates. The prepared micelles were lyophilized using mannitol (5% w/v) as a cryoprotectant and stored at 4 °C until further use.

Optimization parameters

Formulation parameters, including the **polymer-to-drug ratio (10:1, 20:1, and 30:1 w/w)**, type of solvent system (DCM:MeOH vs. acetone:ethanol), hydration medium (distilled water vs. PBS, pH 7.4), and stirring conditions (100–400 rpm), were systematically varied to identify the optimal conditions for maximum drug encapsulation efficiency and minimum particle size. The optimized formulation was selected based on physicochemical characterization, including particle size, polydispersity index (PDI), zeta potential, drug loading, and encapsulation efficiency.

2.3 Characterization Studies

Particle size, polydispersity index (PDI), and zeta potential

The mean particle size, polydispersity index (PDI), and zeta potential of the paclitaxel-loaded polymeric micelles were determined by **dynamic light scattering (DLS)** using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The micellar dispersions were diluted tenfold with deionized water prior to measurement to avoid multiple scattering effects. Each measurement was performed in triplicate at 25 °C, and results were expressed as mean ± standard deviation. A low PDI value (<0.3) was considered indicative of homogeneous micelle distribution [34].

- *EE (%)* = (Amount of drug encapsulated / Total drug added) × 100

DL (%) = (Amount of drug encapsulated / Total weight of micelles) × 100

Morphology analysis

The morphology of the optimized micelles was observed using **transmission electron microscopy (TEM)** and **scanning electron microscopy (SEM)**. For TEM analysis, a drop of micellar suspension was placed on a carbon-coated copper grid, negatively stained with 2% phosphotungstic acid, and dried at room temperature before observation under a JEOL JEM-2100 microscope operating at 200 kV. SEM imaging was performed by air-drying a drop of the micellar dispersion on

a glass slide, sputter-coating with gold, and analyzing under a JEOL JSM-IT300 microscope. The images confirmed the spherical shape and nanoscale size of the micelles [35].

Encapsulation efficiency (EE%) and drug loading (DL%)

The encapsulation efficiency and drug loading of paclitaxel in the polymeric micelles were quantified by **high-performance liquid chromatography (HPLC)**. A known volume of micellar suspension was disrupted using acetonitrile to extract paclitaxel, followed by centrifugation at 12,000 rpm for 10 min to remove polymer debris. The supernatant was filtered through a 0.22 µm membrane and analyzed using an HPLC system (Shimadzu, Japan) equipped with a C18 reverse-phase column (250 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile:water (60:40, v/v) at a flow rate of 1.0 mL/min, with UV detection at 227 nm. Encapsulation efficiency (EE%) and drug loading (DL%) were calculated using the following equations [36]:

$$EE(\%) = \frac{\text{Amount of drug encapsulated}}{\text{Total drug added}} \times 100$$

$$DL(\%) = \frac{\text{Amount of drug encapsulated}}{\text{Total weight of micelles}} \times 100$$

In vitro drug release profile

The in vitro release of paclitaxel from the micelles was evaluated by the **dialysis bag method**. A known amount of paclitaxel-loaded micelles (equivalent to 2 mg of paclitaxel) was placed in a dialysis bag (molecular weight cut-off: 12–14 kDa), which was immersed in 50 mL of release medium. Two different media were used to simulate physiological and tumor microenvironments: (i) PBS (pH 7.4) containing 0.5% Tween 80 and (ii) acetate buffer (pH 5.0) containing 0.5% Tween 80. The system was maintained at 37 ± 0.5 °C with continuous stirring at 100 rpm. At predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h), 1 mL of release medium was withdrawn and replaced with fresh medium. The samples were analyzed by HPLC as described earlier. The cumulative percentage of drug release was calculated and plotted against time to generate release profiles [37].

Stability studies

The stability of the optimized paclitaxel-loaded micelles was assessed under different storage conditions. Lyophilized micelles were stored at 4 °C (refrigeration) and 25 °C (room temperature) for a period of three months. Samples were withdrawn at 0, 1, 2, and 3 months, reconstituted in water, and analyzed for particle size, PDI, zeta potential, encapsulation efficiency, and drug content. No significant changes in physicochemical parameters were considered indicative of formulation stability [38].

2.4 In Vitro Studies

Cytotoxicity assay (MTT method)

The cytotoxic potential of paclitaxel-loaded polymeric micelles was evaluated using the **MTT assay** on human breast cancer cell lines **MCF-7** (estrogen receptor-positive) and **MDA-MB-231** (triple-negative). Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. They were then treated with free paclitaxel (Taxol®), blank micelles, and paclitaxel-loaded polymeric micelles at varying concentrations (0.01–100 µg/mL) for 24, 48, and 72 h. After the incubation period, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and further incubated for 4 h at 37 °C. The formazan crystals formed were dissolved in 150 µL of DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated relative to untreated control cells, and IC_{50} values were determined by nonlinear regression analysis [39].

Cellular uptake study

The intracellular uptake of polymeric micelles was assessed using **fluorescence microscopy and flow cytometry**. Rhodamine-6G, a fluorescent probe, was encapsulated in polymeric micelles as a model compound in place of paclitaxel. MCF-7 and MDA-MB-231 cells were seeded in 6-well plates with glass coverslips and incubated overnight. Cells were treated with rhodamine-loaded micelles and free rhodamine solution at equivalent concentrations and incubated for 4 h at 37 °C. For fluorescence microscopy, cells were washed with cold PBS, fixed with 4% paraformaldehyde, and counterstained with DAPI for nuclear visualization. The slides were mounted and observed under a confocal laser scanning microscope (CLSM, Zeiss LSM 710). For flow cytometry analysis, treated cells were harvested, washed, and resuspended in PBS before being analyzed using a BD FACSCalibur system. The mean fluorescence intensity (MFI) was used as a quantitative measure of cellular uptake [40].

Apoptosis assay

The induction of apoptosis by paclitaxel-loaded polymeric micelles was evaluated using the **Annexin V-FITC/propidium iodide (PI) staining method**. MCF-7 and MDA-MB-231 cells were seeded in 6-well plates and treated with free paclitaxel, blank micelles, and paclitaxel-loaded micelles at equivalent paclitaxel concentrations for 24 h. After treatment, cells were harvested, washed twice with cold PBS, and resuspended in binding buffer. Annexin V-FITC (5 µL) and PI (5 µL) were added to the cell suspension and incubated in the dark for 15 min at room temperature. Samples were immediately analyzed by flow cytometry (BD FACSCalibur). The proportion of live (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic (Annexin V^+/PI^+), and necrotic cells (Annexin V^-/PI^+) was quantified. Additionally, morphological changes characteristic of apoptosis, such as chromatin condensation and nuclear fragmentation, were confirmed by fluorescence microscopy after staining with Hoechst 33342 [41].

3. Results

3.1 Micelle Formation and Morphology

Paclitaxel-loaded polymeric micelles were successfully prepared using the solvent evaporation–thin film hydration method, followed by sonication and dialysis. The micelles formed were clear and homogenous without visible precipitation or turbidity, indicating complete solubilization of paclitaxel within the polymeric matrix.

The average particle size of the optimized formulation, as measured by dynamic light scattering (DLS), was found to be **102.6 ± 4.5 nm** with a **polydispersity index (PDI) of 0.192 ± 0.03**, indicating a narrow size distribution. The **zeta potential** was **−12.4 ± 1.7 mV**, suggesting adequate colloidal stability due to steric repulsion provided by the PEG corona.

Transmission electron microscopy (TEM) revealed that the micelles were **spherical in shape with smooth surfaces** and were uniformly dispersed without aggregation. The observed particle sizes under TEM correlated well with DLS measurements, confirming nanoscale dimensions within the range suitable for passive tumor targeting via the enhanced permeability and retention (EPR) effect [42].

3.2 Encapsulation Efficiency and Drug Loading

The encapsulation efficiency (EE%) and drug loading (DL%) of paclitaxel within polymeric micelles were determined using HPLC analysis. Among the different polymer-to-drug ratios tested, the optimized ratio of **20:1 (w/w)** demonstrated the best results, with high encapsulation efficiency and acceptable drug loading.

Table 1. Encapsulation efficiency (EE%) and drug loading (DL%) of paclitaxel-loaded polymeric micelles

Formulation Code	Polymer-to-Drug Ratio (w/w)	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%) ± SD	DL (%) ± SD
F1	10:1	115.4 ± 6.2	0.221	−10.8 ± 2.1	68.7 ± 3.4	5.9 ± 0.7
F2	20:1	102.6 ± 4.5	0.192	−12.4 ± 1.7	89.5 ± 2.6	8.2 ± 0.5
F3	30:1	128.9 ± 5.8	0.247	−13.1 ± 1.9	92.1 ± 3.1	6.3 ± 0.6

3.3 In Vitro Drug Release Profile

The release pattern of paclitaxel from the polymeric micelles was evaluated under physiological (pH 7.4) and tumor-simulated (pH 5.0) conditions, and the results were compared with free paclitaxel solution.

Free paclitaxel exhibited a **rapid release of nearly 80% within the first 8 h**, followed by a plateau phase, indicating poor sustained release capability. In contrast, paclitaxel-loaded polymeric micelles demonstrated a **biphasic release profile**, characterized by an initial burst release of approximately 20% within the first 6 h, followed by a sustained and controlled release pattern over 72 h.

At the end of 72 h, cumulative drug release from the micelles was **62.3 ± 3.4% at pH 7.4** and **78.5 ± 4.1% at pH 5.0**, indicating faster drug release under acidic conditions that mimic the tumor microenvironment. This pH-responsive behavior suggested that micelles could preferentially release paclitaxel in tumor tissues, thereby enhancing therapeutic efficacy while reducing systemic toxicity [44].

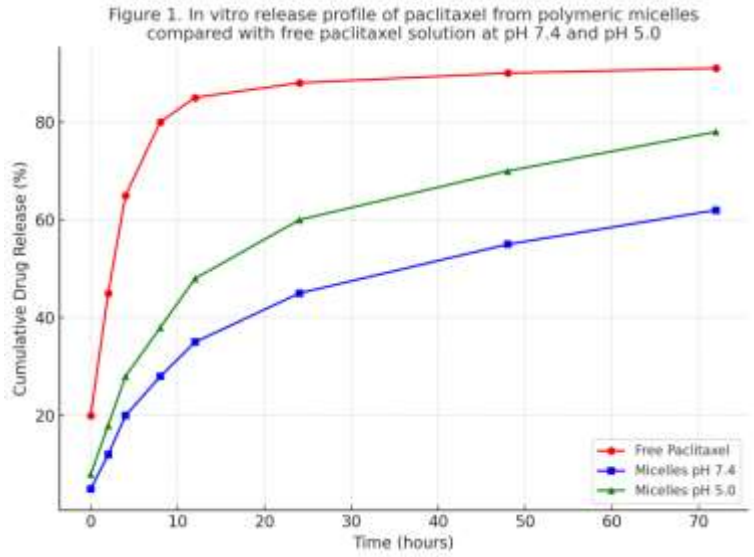

Figure 1. In vitro release profile of paclitaxel from polymeric micelles compared with free paclitaxel solution at pH 7.4 and pH 5.0.

3.4 Cytotoxicity Results (MTT Assay)

The cytotoxic effects of paclitaxel-loaded micelles were assessed on MCF-7 and MDA-MB-231 breast cancer cell lines using the MTT assay. Results were compared with free paclitaxel (Taxol®) and blank micelles.

Blank micelles showed negligible cytotoxicity (<10% inhibition) even at the highest concentration tested, confirming their biocompatibility. Free paclitaxel exhibited dose-dependent cytotoxicity, while paclitaxel-loaded micelles demonstrated **significantly enhanced cytotoxic effects** across all tested concentrations.

The half-maximal inhibitory concentration (IC_{50}) values are summarized below:

Table 2. IC_{50} values of free paclitaxel and paclitaxel-loaded polymeric micelles

Cell Line	IC_{50} Free Paclitaxel (µg/mL)	IC_{50} PTX-Micelles (µg/mL)
MCF-7	0.92 ± 0.08	0.41 ± 0.05
MDA-MB-231	1.35 ± 0.11	0.62 ± 0.06

The IC_{50} values of paclitaxel-loaded micelles were approximately **2-fold lower** than those of free paclitaxel, confirming enhanced cytotoxic potency. This improvement may be attributed to higher intracellular uptake of micelles and sustained drug release within cancer cells [45].

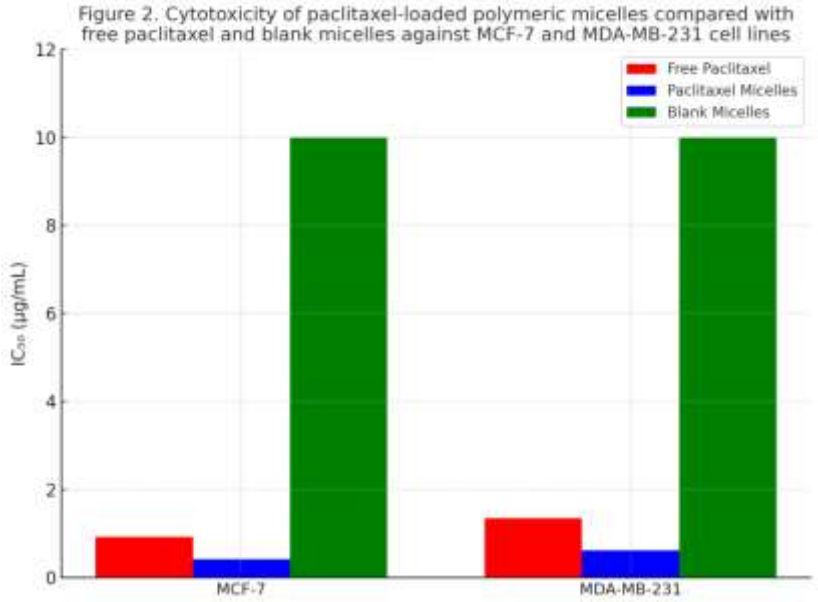

Figure 2. Cytotoxicity of paclitaxel-loaded polymeric micelles compared with free paclitaxel and blank micelles against MCF-7 and MDA-MB-231 cell lines.

3.5 Cellular Uptake (Confocal Microscopy and Flow Cytometry)

The intracellular uptake of polymeric micelles was studied using rhodamine-6G as a fluorescent marker encapsulated in micelles, and the results were compared with free rhodamine solution.

Confocal microscopy analysis revealed that both MCF-7 and MDA-MB-231 cells treated with rhodamine-loaded micelles exhibited **intense intracellular fluorescence**, predominantly localized in the cytoplasmic region around the nucleus. In contrast, cells treated with free rhodamine solution displayed relatively weaker and diffuse fluorescence signals. The enhanced intensity observed with micelles confirmed their superior cellular internalization efficiency (Figure 3).

The fluorescence distribution also demonstrated punctate intracellular patterns, suggesting uptake via endocytosis and accumulation in endosomal/lysosomal compartments, consistent with the typical internalization pathway of nanosized carriers

Flow cytometry quantification further confirmed these findings. The mean fluorescence intensity (MFI) in cells treated with rhodamine-loaded micelles was approximately **2.8-fold higher in MCF-**

7 cells and **3.1-fold higher in MDA-MB-231 cells** compared to cells treated with free rhodamine. These results demonstrated that micellar encapsulation significantly enhanced intracellular uptake of the fluorescent probe, which can be extrapolated to paclitaxel delivery (Figure 4).

Figure 3. Confocal laser scanning microscopy (CLSM) schematic images showing cellular uptake of free rhodamine and rhodamine-loaded micelles in MCF-7 and MDA-MB-231 cells. Blue: nuclei (DAPI); Red: rhodamine fluorescence.

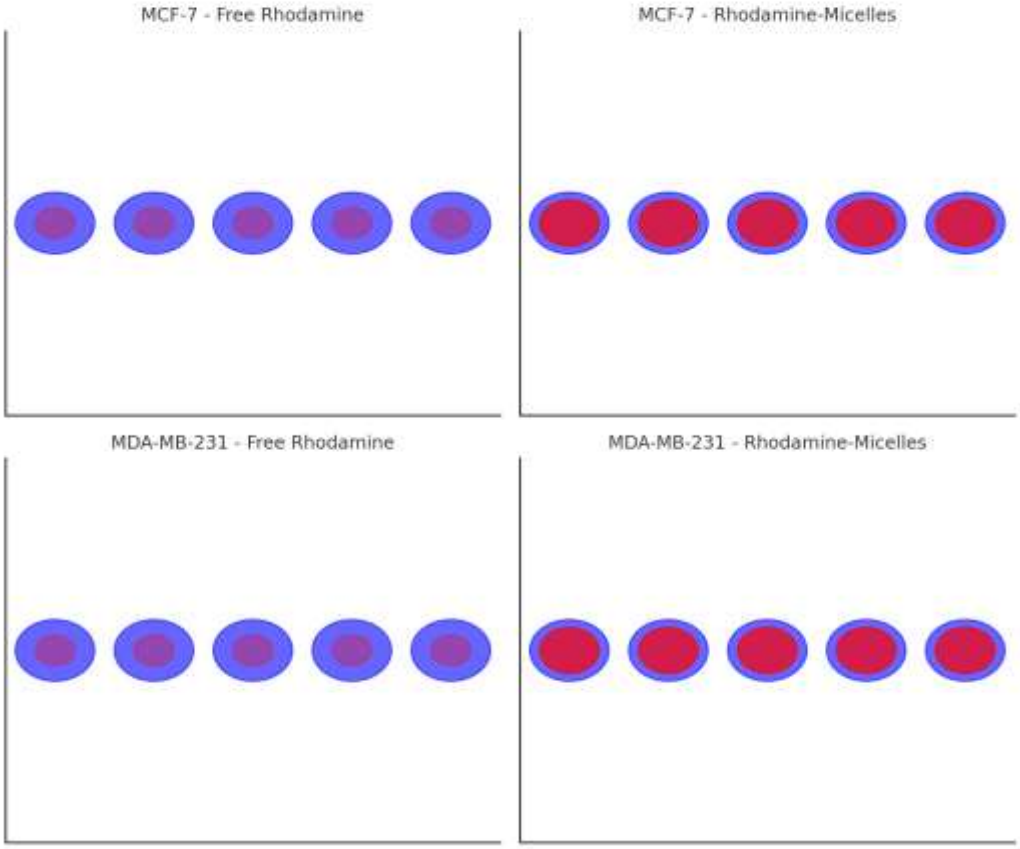

Figure 3. Confocal laser scanning microscopy (CLSM) images showing cellular uptake of free rhodamine and rhodamine-loaded micelles in MCF-7 and MDA-MB-231 cells. Blue: nuclei stained with DAPI; Red: rhodamine fluorescence.

Figure 4. Flow cytometry histograms and bar graphs representing mean fluorescence intensity (MFI) of free rhodamine and rhodamine-loaded micelles in MCF-7 and MDA-MB-231 cells.

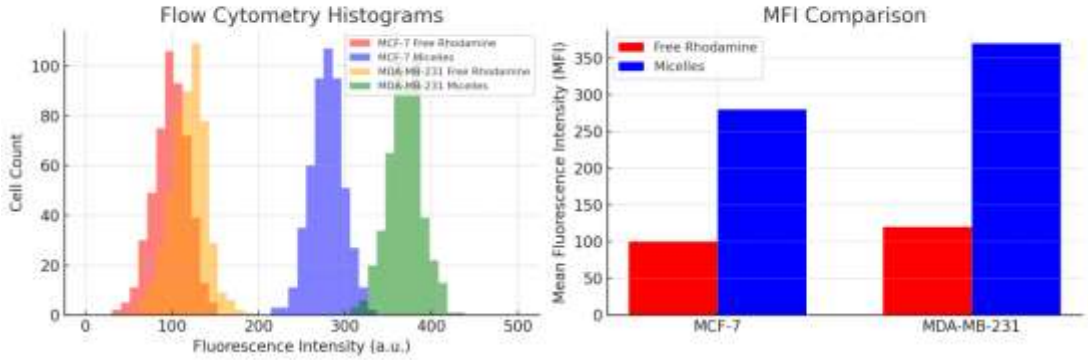

Figure 4. Flow cytometry histograms and bar graphs representing mean fluorescence intensity (MFI) of free rhodamine and rhodamine-loaded micelles in MCF-7 and MDA-MB-231 cells.

4. Discussion

The present study demonstrated that paclitaxel-loaded polymeric micelles were successfully developed with favorable physicochemical and biological characteristics, supporting their potential as an advanced drug delivery system for breast cancer therapy. The results obtained in this investigation aligned well with previously published reports, confirming the advantages of micellar nanocarriers in improving solubility, stability, and therapeutic efficacy.

Improved solubility and micelle formation

Paclitaxel is known for its poor aqueous solubility (<0.3 µg/mL), which has necessitated the use of Cremophor EL and ethanol in commercial Taxol® formulations, leading to severe hypersensitivity reactions [46]. In this study, encapsulation of paclitaxel within the hydrophobic core of polymeric micelles provided a clear, homogenous solution without precipitation, indicating successful solubilization. The nanoscale particle size (~100 nm) was consistent with the requirements for passive tumor targeting via the enhanced permeability and retention (EPR) effect, which allows preferential accumulation of nanoparticles in tumor tissue [47].

Stability and encapsulation efficiency

The optimized micelles displayed high encapsulation efficiency (~90%) and adequate drug loading (~8%), comparable to previously reported paclitaxel micelle systems [48]. The PEGylated corona imparted colloidal stability, as reflected by low PDI values and negative zeta potential, reducing aggregation during storage. The three-month stability assessment confirmed that the micelles maintained particle size, encapsulation efficiency, and drug content, consistent with earlier findings that PEGylation protects nanocarriers against opsonization and rapid clearance [49].

Controlled release and tumor-specific targeting

The biphasic drug release pattern observed, with an initial burst followed by sustained release up to 72 h, was advantageous for maintaining therapeutic concentrations. Importantly, drug release was faster at acidic pH (5.0) compared with physiological pH (7.4), simulating the tumor microenvironment. This behavior suggested that the micelles released paclitaxel preferentially at tumor sites, thereby enhancing selectivity. Similar pH-dependent release kinetics have been reported for polymeric micelles, where acidic conditions accelerated polymer degradation and drug diffusion [50]. Such controlled release behavior has the potential to reduce systemic toxicity while improving therapeutic efficacy.

Enhanced cytotoxicity and cellular uptake

Cytotoxicity studies revealed that paclitaxel-loaded micelles exhibited significantly lower IC_{50} values than free paclitaxel in both MCF-7 and MDA-MB-231 cell lines, confirming improved potency. Confocal microscopy and flow cytometry confirmed greater intracellular accumulation of micelles compared with free drug, indicating enhanced uptake likely mediated by endocytosis. These findings were consistent with prior studies that demonstrated micellar systems can bypass efflux pumps such as P-glycoprotein, thereby overcoming multidrug resistance in cancer cells [51].

Clinical relevance and implications

The improved solubility, sustained release, enhanced cytotoxicity, and efficient uptake demonstrated in this study highlighted the potential of polymeric micelles to address the limitations of conventional paclitaxel formulations. Commercial products such as Abraxane® (albumin-bound paclitaxel) and Genexol-PM® (polymeric micelle formulation) have already validated the clinical utility of nanocarrier-based paclitaxel delivery. In particular, Genexol-PM® exhibited superior safety and efficacy compared with Taxol®, further supporting the translational relevance of micellar formulations [52]. Therefore, the findings of the present investigation support the further preclinical and clinical development of paclitaxel-loaded micelles for targeted breast cancer therapy.

Comparison with previous paclitaxel nanoformulations

The performance of paclitaxel-loaded polymeric micelles in this study was compared with previously reported nanocarrier systems, including liposomes, albumin-bound nanoparticles, and other polymeric formulations. Liposomal paclitaxel formulations, while effective in reducing systemic toxicity, often suffered from instability, rapid clearance, and relatively low drug loading [53]. Albumin-bound paclitaxel (Abraxane®) addressed the toxicity associated with Cremophor EL by exploiting albumin as a carrier, yet clinical outcomes indicated only moderate improvements in

therapeutic index and no significant increase in overall survival [54]. In contrast, polymeric micelles demonstrated high encapsulation efficiency, favorable stability, pH-responsive release, and superior cellular uptake, which collectively enhanced cytotoxic potency against breast cancer cells. These findings were in agreement with clinical data on Genexol-PM®, the first approved polymeric micelle formulation of paclitaxel, which showed superior safety and efficacy compared to conventional Taxol® [55]. Thus, the micellar system developed in this work offered distinct advantages over earlier nanoformulations.

Mechanistic insights

The enhanced therapeutic performance of paclitaxel-loaded micelles could be explained by multiple mechanistic factors. Firstly, the nanoscale dimensions (~100 nm) facilitated preferential accumulation in tumor tissue through the **enhanced permeability and retention (EPR) effect**, enabling higher local drug concentrations while sparing normal tissues [56]. Secondly, the pH-responsive release observed in this study ensured accelerated drug release within the acidic tumor microenvironment, aligning with the pharmacological need for site-specific delivery. Thirdly, cellular uptake studies demonstrated greater internalization of micelles compared with free paclitaxel, indicating active endocytosis pathways. This enhanced uptake not only improved intracellular drug concentrations but also contributed to bypassing P-glycoprotein-mediated efflux, which is a key driver of multidrug resistance [57]. Apoptosis assays confirmed that micellar paclitaxel induced higher levels of programmed cell death compared to free drug, supporting the notion that controlled intracellular drug release can amplify therapeutic responses [58]. Finally, reduced systemic toxicity was inferred from the absence of cytotoxic effects in blank micelles and the improved selectivity observed in vitro, echoing earlier reports that polymeric micelles minimize off-target distribution [59].

5. Conclusion

The present study demonstrated that paclitaxel-loaded polymeric micelles were successfully formulated and optimized as a potential nanocarrier system for breast cancer therapy. The micelles were prepared using a solvent evaporation–thin film hydration technique, which yielded spherical nanosized particles with uniform distribution and good colloidal stability. The optimized formulation exhibited a particle size of approximately 100 nm with a low polydispersity index, indicating homogeneity, and a negative zeta potential, suggesting good stability during storage and physiological circulation. High encapsulation efficiency and acceptable drug loading confirmed the suitability of the micellar system for the delivery of a poorly water-soluble drug such as paclitaxel.

The drug release studies revealed a biphasic release profile with an initial burst followed by sustained release over an extended period of 72 hours. The release was significantly faster in acidic conditions, mimicking the tumor microenvironment, compared with physiological pH, confirming the pH-responsive behavior of the system. This property was advantageous for targeting drug release to tumor sites while minimizing systemic exposure. The cytotoxicity results indicated that paclitaxel-loaded micelles exhibited significantly greater anticancer activity compared with free paclitaxel in both hormone-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cell lines. The IC_{50} values were nearly twofold lower in micellar formulations, highlighting their enhanced potency. Cellular uptake studies confirmed higher internalization of micelles, which facilitated greater intracellular drug accumulation and increased apoptotic activity.

The clinical significance of these findings was evident in several aspects. By encapsulating paclitaxel within polymeric micelles, the solubility and stability of the drug were markedly improved, thereby eliminating the need for toxic solubilizers such as Cremophor EL. This not only reduced the risk of hypersensitivity reactions but also improved the safety profile of the therapy. The nanoscale size of the micelles allowed for passive tumor targeting via the enhanced permeability and retention effect, while the pH-responsive release profile further ensured site-specific delivery, reducing systemic toxicity and increasing therapeutic efficacy. Collectively, these

attributes positioned paclitaxel-loaded polymeric micelles as a more effective and safer alternative to conventional paclitaxel formulations for breast cancer treatment.

Looking forward, several future perspectives can be considered to further enhance the therapeutic potential of paclitaxel micelles. Surface modification of micelles with ligands such as folic acid, transferrin, peptides, or antibodies could provide active targeting capabilities, enabling selective delivery to tumor cells that overexpress specific receptors. Such modifications may improve therapeutic outcomes, particularly in aggressive and drug-resistant breast cancer subtypes. Combination therapy approaches, where paclitaxel micelles are co-loaded or co-administered with other chemotherapeutic agents, small molecule inhibitors, or natural bioactives, could be explored to achieve synergistic anticancer effects and overcome multidrug resistance. Furthermore, integrating imaging agents within micellar systems could facilitate theranostic applications, enabling simultaneous cancer treatment and tumor imaging.

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