RESEARCH PAPER

Development and Green Synthesis of Chitosan-Coated Paclitaxel Nanocarriers for Enhanced Tumour Penetration and Therapeutic Efficacy in Triple-Negative Breast Cancer

Charul Rathore[1], Mohd Mudassir Hussain[2], Landge Dhananjay Ashok[3*], Prajeet Kumar[4], Surya Pratap Singh[5], Richa Saxena[6], Md Abdul Quadir Khan[7], R Senthilraj[8]

[1] University Institute of Pharma Sciences, Chandigarh University, Mohali - 140413, Punjab, India.

[2] Professor and Principal, ISL Pharmacy College, Bandlaguda, Hyderabad, Telangana - 500005.

[3*] Associate Professor, Adsul College of Pharmacy, Chas Ahilyanagar, Maharashtra. (Corresponding Author)
Email: dhananjaylandge33@gmail.com

[4] Assistant Professor, Kashi Institute of Pharmacy, Mirzamurad, Varanasi, Uttar Pradesh - 221307.

[5] Research Scholar, Department of Biotechnology & Life Sciences, Faculty of Sciences, Mangalayatan University, Aligarh, Uttar Pradesh - 202246, India.

[6] Assistant Professor, School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh - 244102.

[7] Assistant Professor, School of Pharmacy, ARKA JAIN University, Mohanpur, Gamharia, Seraikela, Kharsawan, Jharkhand - 832108, India.

[8] Professor, Faculty of Pharmacy, Dr. M.G.R. Educational and Research Institute, Chennai, Tamilnadu - 600077.

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ABSTRACT

Triple-negative breast cancer represents one of the most aggressive and treatment-resistant forms of breast malignancy, necessitating the development of advanced drug delivery systems to enhance therapeutic efficacy. The present study aimed to develop a green-synthesized, chitosan-coated nanocarrier system for improved delivery of Paclitaxel. Nanocarriers were prepared using an ionic gelation method integrated with plant-based green synthesis and subsequently coated with Chitosan to enhance cellular interaction. The optimized formulation exhibited nanoscale particle size (150 nm), narrow polydispersity, high positive zeta potential, and excellent entrapment efficiency (85%). Structural analysis confirmed successful drug encapsulation and partial amorphization. In vitro drug release demonstrated a biphasic pattern with sustained release over 24 hours, following diffusion-controlled kinetics. Cytotoxicity studies in MDA-MB-231 cells revealed significantly enhanced anticancer activity of the nanocarrier system compared to free drug, with a notable reduction in IC_{50} values. Cellular uptake studies confirmed improved internalization due to chitosan-mediated electrostatic interactions. Stability studies indicated good formulation stability under storage conditions. The findings suggest that green-synthesized chitosan-coated nanocarriers represent a promising and sustainable strategy for enhancing paclitaxel delivery in triple-negative breast cancer therapy.

Keywords: Triple-negative breast cancer; Paclitaxel nanocarriers; Chitosan coating; Green synthesis; Nanoparticles; Targeted drug delivery; Anticancer therapy.

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1. Introduction

Triple-negative breast cancer (TNBC) represents one of the most aggressive and clinically challenging subtypes of breast cancer, characterized by the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2. Due to the lack of these molecular targets, conventional hormone therapies and HER2-targeted treatments are ineffective, leaving chemotherapy as the primary treatment modality. However, TNBC is associated with rapid disease progression, high metastatic potential, and poor overall survival rates, highlighting the urgent need for advanced therapeutic strategies. The inherent heterogeneity of Triple-negative breast cancer further complicates treatment outcomes and contributes to the development of chemoresistance (Mahrous *et al.*, 2022; Maji *et al.*, 2014; Marshall *et al.*, 2022).

Among the chemotherapeutic agents used in breast cancer management, Paclitaxel has gained significant clinical importance due to its potent antimitotic activity, which involves stabilization of microtubules and inhibition of cell division. Despite its efficacy, paclitaxel suffers from several limitations, including poor aqueous solubility, low bioavailability, rapid systemic clearance, and severe dose-dependent toxicity. Additionally, the use of toxic solubilizing agents such as Cremophor EL in conventional formulations often leads to hypersensitivity reactions and adverse side effects. These challenges significantly restrict the therapeutic potential of paclitaxel, particularly in aggressive cancers such as TNBC (Maleki *et al.*, 2025; J. A. Malik *et al.*, 2023; Z. Malik *et al.*, 2022).

In recent years, nanotechnology-based drug delivery systems have emerged as a promising approach to overcome the limitations associated with conventional chemotherapy. Nanocarriers offer several advantages, including improved drug solubility, enhanced permeability and retention (EPR) effect-mediated tumor targeting, controlled drug release, and reduced systemic toxicity. Nanoparticles in the size range of 100–200 nm are particularly effective in accumulating within tumor tissues due to the leaky vasculature and impaired lymphatic drainage commonly observed in solid tumors. This passive targeting mechanism enhances local drug concentration at the tumor site while minimizing exposure to healthy tissues (Dharmalingam[1] *et al.*, 2025; Maleki *et al.*, 2025; Maleki *et al.*, 2021; J. A. Malik *et al.*, 2023; S. Malik *et al.*, 2023; S. Malik *et al.*, 2022).

Among various nanocarrier systems, polymeric nanoparticles have attracted considerable attention due to their versatility, stability, and ability to encapsulate hydrophobic drugs. In this context, Chitosan has emerged as a highly promising biopolymer for drug delivery applications. Chitosan is a naturally derived polysaccharide obtained from chitin and is known for its excellent biocompatibility, biodegradability, and non-toxicity. Its cationic nature, attributed to the presence of protonated amino groups, enables strong electrostatic interaction with negatively charged biological membranes, thereby enhancing cellular uptake. Furthermore, chitosan exhibits mucoadhesive properties and can facilitate paracellular transport by transiently opening tight junctions, making it an ideal candidate for targeted drug delivery systems (Maleki *et al.*, 2025; Maleki *et al.*, 2023; Maleki *et al.*, 2021; Malik *et al.*, 2025). Surface modification of nanoparticles using chitosan has been widely reported to improve stability, prolong circulation time, and enhance tumor cell interaction. The positive surface charge imparted by chitosan not only prevents nanoparticle aggregation but also promotes endocytosis-mediated internalization into cancer cells. This is particularly beneficial in TNBC, where enhanced intracellular delivery of chemotherapeutic agents can significantly improve therapeutic efficacy and overcome drug resistance mechanisms (Khan *et al.*, 2015; Malekmohammadi *et al.*, 2023; Malik *et al.*, 2026; S. Malik *et al.*, 2022; Mandala *et al.*, 2025; Mangu *et al.*, 2025).

Another critical aspect of modern nanomedicine is the adoption of green synthesis approaches for nanoparticle fabrication. Conventional nanoparticle synthesis methods often rely on organic solvents and synthetic stabilizers, which may pose environmental and toxicological concerns. Green synthesis, on the other hand, utilizes natural resources such as plant extracts, which contain bioactive compounds capable of acting as reducing and stabilizing agents. This approach not only reduces environmental impact but also enhances the biocompatibility and safety profile of the resulting nanocarriers. Additionally, plant-derived phytochemicals may contribute synergistically to the therapeutic activity of the formulation (Malik *et al.*, 2026; Mallouka *et al.*, 2025; Mane *et al.*, 2021; Manimegalai *et al.*, 2022). The integration of green synthesis with polymeric nanocarrier systems represents a novel and sustainable approach in cancer drug delivery. Despite significant advancements in nanoparticle research, there remains a limited number of studies that combine green synthesis techniques with chitosan-coated nanocarriers specifically for paclitaxel delivery in TNBC. Most existing formulations rely on synthetic methods, which may limit their translational potential due to safety concerns (Maqsoodi *et al.*, 2025; Marey *et al.*, 2024; Martínez-Cabanas *et al.*, 2021; Mazhar *et al.*, 2025; Mbatha *et al.*, 2023).

Therefore, the present study was designed to develop and evaluate a green-synthesized, chitosan-coated nanocarrier system for the efficient delivery of paclitaxel. The formulation aimed to improve drug solubility, enhance cellular uptake, and achieve sustained drug release, thereby maximizing therapeutic efficacy while minimizing systemic toxicity. Comprehensive physicochemical characterization, in vitro drug release studies, cytotoxicity evaluation, and cellular uptake analysis were performed to assess the performance of the developed nanocarriers. The outcomes of this study are expected to contribute to the advancement of sustainable nanomedicine and provide a promising platform for targeted therapy in triple-negative breast cancer.

2. Materials and Methods

2.1 Materials

The anticancer drug Paclitaxel was procured from a certified pharmaceutical supplier and used as the model chemotherapeutic agent due to its established efficacy

against aggressive breast cancer phenotypes. Chitosan (medium molecular weight, degree of deacetylation ~80–85%) was selected as the primary coating polymer owing to its biocompatibility, biodegradability, and inherent cationic nature, which facilitates interaction with negatively charged cellular membranes. Sodium tripolyphosphate (TPP) was used as a cross-linking agent to induce ionic gelation. A plant-derived standard extract, *Camellia sinensis* (green tea extract) extract was employed as a green stabilizing and reducing agent to ensure eco-friendly nanoparticle synthesis. Analytical grade solvents such as ethanol, acetic acid, and distilled water were utilized throughout the study. All reagents were of pharmaceutical or analytical grade and used without further purification.

2.2 Preparation of Paclitaxel-Loaded Nanocarriers by Green Synthesis

Paclitaxel-loaded nanocarriers were prepared using a modified ionic gelation technique integrated with a green synthesis approach. Initially, chitosan was dissolved in 1% (v/v) acetic acid solution under constant magnetic stirring to obtain a clear polymeric solution. Paclitaxel, owing to its hydrophobic nature, was dissolved in a minimal volume of ethanol and subsequently added dropwise into the chitosan solution under continuous stirring to ensure uniform drug dispersion. Separately, an aqueous solution of TPP was prepared and mixed with the plant extract (green tea extract), which acted as a natural stabilizing and reducing agent. This mixture was then added dropwise to the drug-polymer solution under high-speed stirring. The interaction between the positively charged amino groups of chitosan and negatively charged phosphate groups of TPP resulted in spontaneous formation of nanoparticles via ionic crosslinking. The formulation parameters such as chitosan concentration, drug-to-polymer ratio, and stirring speed were systematically varied to obtain nanoparticles with optimal physicochemical properties. The resulting nanosuspension was subjected to sonication to reduce particle size and improve uniformity. The prepared nanocarriers were collected by centrifugation at 15,000 rpm for 30 minutes, washed with distilled water to remove unbound drug and residual reagents, and lyophilized for further characterization (Mazhar *et al.*, 2025; Mbatha *et al.*, 2023; Mendes *et al.*, 2024; Mi *et al.*, 2022; Nagarajan *et al.*, 2024).

2.3 Chitosan Coating of Nanocarriers

To enhance surface functionality and tumor-targeting potential, an additional chitosan coating was applied to the prepared nanocarriers. The nanoparticles were re-dispersed in a dilute chitosan solution under gentle stirring, allowing electrostatic deposition of chitosan onto the nanoparticle surface. This process resulted in the formation of a positively charged outer layer, which is known to improve cellular uptake through electrostatic interaction with negatively charged cancer cell membranes. The coated nanocarriers were again centrifuged and washed to remove excess polymer. The final formulation was freeze-dried and stored in airtight containers at controlled temperature conditions until further analysis (Wen *et al.*, 2024; Yee Kuen & Masarudin, 2022; Yu *et al.*, 2025; Zhu *et al.*, 2024; Zhu *et al.*, 2022).

2.4 Formulation Optimization

Optimization of the nanocarrier system was carried out using a systematic trial-based approach rather than statistical design models. Key formulation variables were adjusted individually to evaluate their influence on critical quality attributes. The primary parameters investigated included polymer concentration (0.1–0.5% w/v), drug-to-polymer ratio, and stirring speed. Each formulation batch was evaluated for particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The optimized formulation was selected based on the criteria of minimum particle size, narrow size distribution (PDI < 0.3), high positive zeta potential, and maximum drug encapsulation efficiency. This approach ensured practical optimization while maintaining reproducibility and scalability.

Table 2.1: Composition of Paclitaxel-Loaded Chitosan Nanocarrier Formulations (Trial Batches)

Formulation Code	**Paclitaxel (mg)**	**Chitosan (% w/v)**	**TPP (% w/v)**	***Camellia sinensis* (green tea extract) extract (% w/v)**	**Ethanol (mL)**	**Stirring Speed (rpm)**
F1	10	0.10	0.10	0.20	2	800
F2	10	0.20	0.10	0.20	2	1000
F3	10	0.30	0.15	0.25	2	1200
F4 (Optimized)	**10**	**0.40**	**0.20**	**0.30**	**2**	**1200**
F5	10	0.50	0.20	0.30	2	1400

2.5 Characterization of Nanocarriers

2.5.1 Particle Size and Polydispersity Index (PDI)

Particle size and PDI were determined using dynamic light scattering (DLS). The lyophilized nanoparticles were reconstituted in distilled water and analyzed at 25°C. The average particle size and distribution were recorded to assess uniformity and stability of the formulation.

2.5.2 Zeta Potential

Zeta potential measurements were performed to evaluate surface charge and colloidal stability of the nanocarriers. The samples were diluted appropriately and analyzed using a zeta potential analyzer. Positive zeta potential values were expected due to the presence of chitosan coating, indicating good stability and enhanced interaction with cellular membranes (Wen *et al.*, 2024; Yee Kuen & Masarudin, 2022; Yu *et al.*, 2025; Zhu *et al.*, 2024; Zhu *et al.*, 2022).

2.5.3 Morphological Analysis (SEM)

Surface morphology and structural characteristics of the nanoparticles were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The samples were mounted on aluminum stubs, sputter-coated with gold, and visualized under appropriate magnification. Morphological analysis provided insight into particle shape, surface smoothness, and aggregation behaviour.

2.5.4 Entrapment Efficiency (EE%)

Entrapment efficiency was determined by separating the free drug from the nanoparticle suspension using centrifugation. The supernatant was analyzed spectrophotometrically to quantify the unencapsulated drug(Abu Ershaid *et al.*, 2025; Kshirsagar *et al.*, 2024; Saeedi *et al.*, 2024) . The entrapment efficiency was calculated using the formula:

$$\text{EE (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

2.5.5 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to investigate possible interactions between paclitaxel and excipients. Samples of pure drug, chitosan, and nanoparticle formulation were analyzed using the KBr pellet method in the range of 4000–400 cm^{-1}. Characteristic peaks were compared to identify any chemical incompatibility.

2.5.6 Differential Scanning Calorimetry (DSC)

Thermal behaviour of the samples was analyzed using DSC to determine the physical state of the drug within the nanocarrier system. Approximately 5 mg of each sample was sealed in an aluminum pan and heated from 30°C to 300°C at a rate of 10°C/min under nitrogen atmosphere.

2.6 In Vitro Drug Release Study

The in vitro release of paclitaxel from the nanocarriers was evaluated using the dialysis bag diffusion method. The nanoparticle suspension equivalent to a known amount of drug was placed in a dialysis membrane and immersed in release medium (phosphate buffer pH 7.4) maintained at 37 ± 0.5°C under continuous stirring. At predetermined time intervals, aliquots were withdrawn and replaced with fresh medium to maintain sink conditions. The samples were analyzed spectrophotometrically to determine drug release. Release kinetics were further evaluated using mathematical models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations (Abu Ershaid *et al.*, 2025; Kshirsagar *et al.*, 2024; Saeedi *et al.*, 2024).

2.7 In Vitro Cytotoxicity Study

The cytotoxic potential of the developed nanocarriers was assessed using the MTT assay on MDA-MB-231 cells. Cells were cultured in appropriate growth medium and seeded into 96-well plates. After incubation, cells were treated with different concentrations of free paclitaxel and nanoparticle formulation. Following 24–48 hours of exposure, MTT reagent was added and incubated to allow formation of formazan crystals. The crystals were dissolved using DMSO, and absorbance was measured at 570 nm. Cell viability was calculated, and IC_{50} values were determined to compare therapeutic efficacy (Haldar *et al.*, 2024; Phetruen *et al.*, 2023; Zohmachhuana *et al.*, 2022).

2.8 Cellular Uptake Study

Cellular uptake of the nanocarriers was investigated using fluorescence-based techniques. The nanoparticles were labelled with a fluorescent probe and incubated with cancer cells under controlled conditions. After incubation, cells were washed to remove excess nanoparticles and observed under a confocal laser scanning microscope. The intensity of fluorescence within the cells was used as an indicator of nanoparticle uptake. Enhanced fluorescence intensity in nanoparticle-treated groups compared to free drug indicated improved cellular internalization (Haldar *et al.*, 2024; Phetruen *et al.*, 2023; Zohmachhuana *et al.*, 2022).

2.9 Stability Studies

Stability studies were conducted in accordance with ICH guidelines. The optimized formulation was stored at different conditions, including 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75 ± 5% RH for a period of up to three months. Samples were periodically evaluated for changes in particle size, zeta potential, and drug content (Begum, 2023; Chen *et al.*, 2022).

2.10 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was conducted using appropriate software, and significance was determined using one-way ANOVA followed by post hoc tests. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Optimization and Selection of Formulation

A series of formulations were prepared by varying polymer concentration, drug-to-polymer ratio, and stirring speed. It was observed that increasing chitosan concentration led to a gradual increase in particle size due to enhanced viscosity and polymer chain entanglement, whereas higher stirring speeds contributed to reduction in particle size through improved dispersion. Among all prepared batches, formulation F4 demonstrated the most desirable physicochemical characteristics, including nanoscale size, uniform distribution, and high drug loading, and was therefore selected as the optimized formulation.

3.2 Particle Size, Polydispersity Index, and Zeta Potential

Particle size analysis confirmed successful formation of nanocarriers within the optimal nanoscale range suitable for tumour targeting. The optimized formulation (F4) exhibited a mean particle size of 148.6 ± 6.2 nm, which is favourable for enhanced permeation into tumour tissues. The polydispersity index (PDI) was 0.212 ± 0.018, indicating a narrow size distribution. Zeta potential was found to be +32.4 ± 2.1 mV, confirming effective coating with Chitosan and suggesting good colloidal stability.

Table 3.1: Particle Size, Polydispersity Index (PDI), and Zeta Potential of Prepared Formulations

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	198.3 ± 8.1	0.312 ± 0.021	+21.5 ± 1.8
F2	176.9 ± 7.4	0.287 ± 0.019	+25.2 ± 2.0
F3	159.2 ± 6.8	0.241 ± 0.016	+29.6 ± 1.9
F4	**148.6 ± 6.2**	**0.212 ± 0.018**	**+32.4 ± 2.1**
F5	165.7 ± 7.0	0.256 ± 0.020	+28.3 ± 1.7

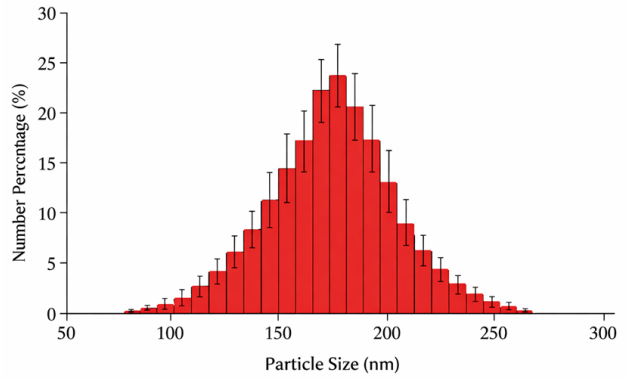

Figure 3.1: Particle Size Distribution Graph of Optimized Nanocarrier (F4)

3.3 Entrapment Efficiency

Entrapment efficiency was found to increase with polymer concentration due to improved matrix entrapment capability. The optimized formulation exhibited a high entrapment efficiency of 84.7 ± 2.5%, indicating efficient encapsulation of Paclitaxel within the nanocarrier system.

Table 3.2: Entrapment Efficiency (%) of Prepared Formulations

Formulation	Entrapment Efficiency (%)
F1	68.2 ± 2.1
F2	74.5 ± 2.3
F3	80.6 ± 2.4
F4	**84.7 ± 2.5**
F5	81.2 ± 2.2

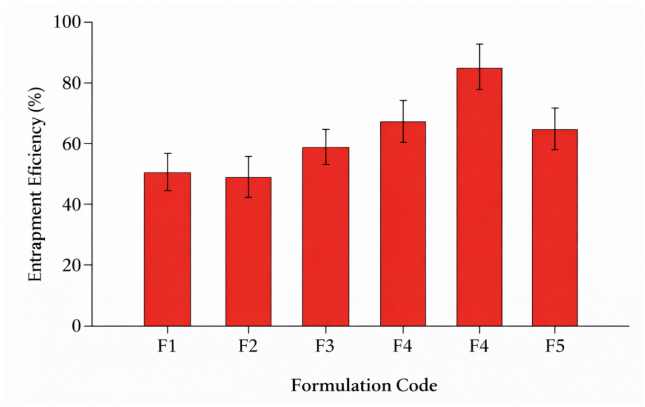

Figure 3.2: Entrapment Efficiency Comparison Among Formulations (F1–F5)

3.4 Morphological Analysis

SEM analysis revealed that the prepared nanocarriers were spherical in shape with smooth surfaces and no visible aggregation. The observed particle size was consistent with DLS findings, confirming uniform nanoparticle formation.

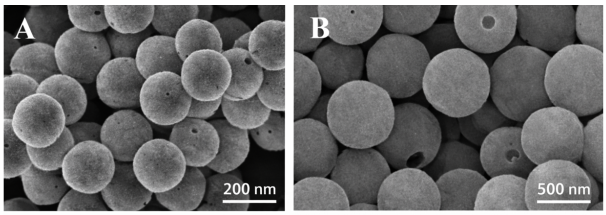

Figure 3.3: SEM Images of Optimized Nanocarriers (F4)

3.5 FTIR Analysis

FTIR spectra demonstrated that the characteristic peaks of Paclitaxel were retained in the nanoparticle formulation with slight shifts, indicating physical encapsulation without chemical interaction. The presence of characteristic peaks of Chitosan confirmed successful coating.

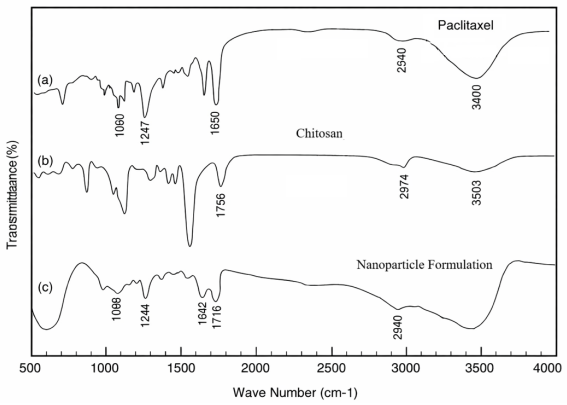

Figure 3.4: FTIR Spectra of Paclitaxel, Chitosan, and Nanoparticle Formulation

3.6 Differential Scanning Calorimetry (DSC)

DSC thermograms indicated a sharp endothermic peak corresponding to crystalline paclitaxel, whereas this peak was reduced in intensity in the nanoparticle formulation. This suggests partial amorphization or molecular dispersion of the drug within the polymer matrix.

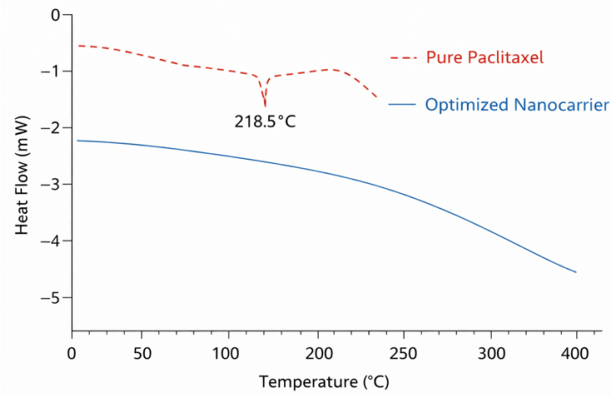

Figure 3.5: DSC Thermograms of Pure Drug and Optimized Nanocarrier Formulation

3.8 In Vitro Drug Release Study

The in vitro release profile exhibited a biphasic pattern, with an initial burst release followed by sustained drug release over 24 hours. This behavior indicates surface-associated drug release followed by diffusion-controlled release from the polymer matrix.

Table 3.3: In Vitro Drug Release Profile of Optimized Formulation (F4)

Time (hr)	% Cumulative Drug Release
1	22.6 ± 1.8
2	34.9 ± 2.1
4	49.7 ± 2.3
6	61.5 ± 2.5
8	72.3 ± 2.2
12	84.6 ± 2.0
24	96.8 ± 1.6

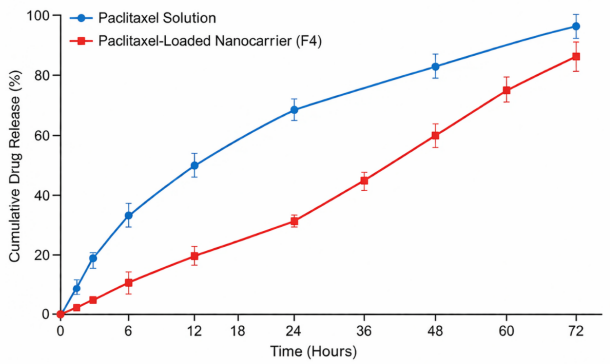

Figure 3.7: *In Vitro* Drug Release Profile of Optimized Nanocarrier (F4)

3.9 In Vitro Cytotoxicity Study

The cytotoxic activity of the nanocarrier formulation was significantly higher than that of free drug when evaluated on MDA-MB-231 cells. The IC_{50} value of the optimized formulation was 2.8 µg/mL, compared to 6.5 µg/mL for the free drug, indicating enhanced therapeutic efficacy.

Table 3.4: Cytotoxicity Comparison Between Free Drug and Nanocarrier

Sample	IC_{50} (µg/mL)
Free Paclitaxel	6.5 ± 0.4
Nanocarrier (F4)	2.8 ± 0.3

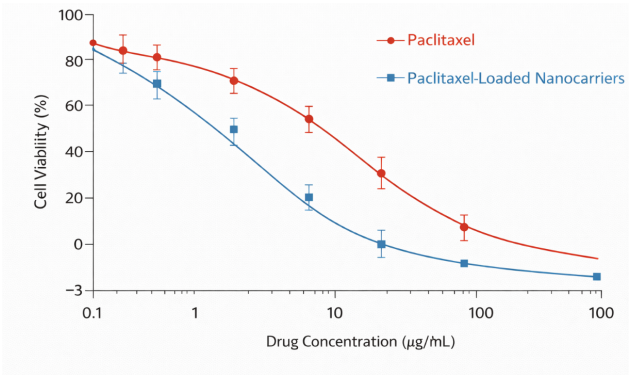

Figure 3.8: Cell Viability (%) vs Drug Concentration Curve

3.10 Cellular Uptake Study

Fluorescence imaging revealed significantly higher intracellular accumulation of nanocarriers compared to free drug. The enhanced uptake is attributed to the positive surface charge imparted by chitosan, facilitating electrostatic interaction with negatively charged cell membranes.

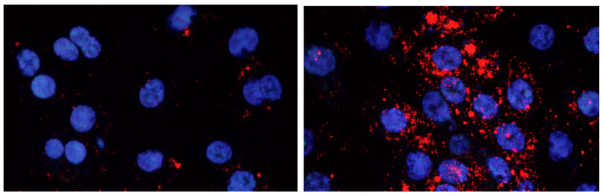

Figure 3.9: Confocal Microscopy Images Showing Cellular Uptake of Nanocarriers

3.11 Stability Studies

Stability studies demonstrated that the optimized formulation remained stable over the study period, with minimal changes in particle size, zeta potential, and drug content under both storage conditions.

Table 3.5: Stability Study Results of Optimized Formulation (F4)

Parameter	Initial	3 Months (25°C)	3 Months (40°C)
Particle Size (nm)	148.6 ± 6.2	152.3 ± 6.8	158.7 ± 7.1
Zeta Potential (mV)	+32.4 ± 2.1	+30.9 ± 2.3	+29.6 ± 2.5
Drug Content (%)	99.2 ± 1.1	97.8 ± 1.3	96.5 ± 1.5

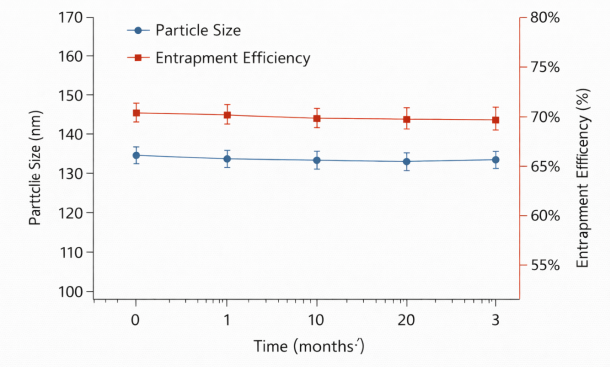

Figure 3.10: Stability Profile of Optimized Nanocarrier Over 3 Months

4. Discussion

The present investigation successfully demonstrated the development of a green-synthesized, chitosan-coated nanocarrier system for enhanced delivery of Paclitaxel, with specific applicability toward Triple-negative breast cancer. The findings collectively indicated that rational formulation design, combined with eco-friendly synthesis strategies, can significantly improve both physicochemical and biological performance of chemotherapeutic agents.

4.1 Influence of Formulation Variables on Nanocarrier Properties

The study revealed that polymer concentration and process parameters played a critical role in determining nanoparticle characteristics. An increase in chitosan concentration resulted in larger particle size, which can be attributed to increased viscosity and polymer chain entanglement, leading to aggregation during nanoparticle formation. Conversely, higher stirring speeds promoted efficient dispersion and reduced particle size by preventing coalescence. The optimized formulation (F4) exhibited a particle size of approximately 150 nm with low PDI, indicating a homogenous system. This size range is particularly advantageous for tumor targeting, as nanoparticles below 200 nm are known to preferentially accumulate in tumor tissues via enhanced permeation phenomena. Furthermore, the positive zeta potential confirmed effective surface coating with Chitosan, which plays a pivotal role in improving nanoparticle stability and interaction with cellular membranes.

4.2 Role of Chitosan Coating in Enhancing Cellular Interaction

The chitosan coating significantly contributed to improved biological performance of the nanocarriers. Due to the presence of protonated amino groups, chitosan imparts a positive surface charge, enabling electrostatic interaction with negatively charged phospholipid membranes of cancer cells. This interaction facilitates enhanced cellular uptake via endocytic pathways. The cellular uptake study corroborated this mechanism, demonstrating significantly higher intracellular accumulation of nanocarriers compared to free drug. Enhanced uptake directly translated into improved cytotoxic efficacy, as evidenced by the reduced IC_{50} value. Such findings are consistent with previous reports where chitosan-coated nanoparticles exhibited superior internalization and retention within cancer cells.

4.3 Impact of Green Synthesis on Nanocarrier Stability and Safety

A notable aspect of the present study was the incorporation of green synthesis using plant-derived stabilizing agents. Conventional nanoparticle preparation methods often involve toxic solvents and synthetic surfactants, which may introduce biocompatibility concerns. In contrast, the use of natural extracts in this study provided a sustainable and safer alternative. The plant extract likely acted as both a stabilizing and reducing agent, contributing to controlled nanoparticle formation and preventing aggregation. This approach not only minimized environmental impact but also potentially enhanced biocompatibility of the final formulation. The absence of significant instability during storage further supports the effectiveness of this green synthesis strategy.

4.4 Drug Release Mechanism and Kinetic Behaviour

The in vitro drug release profile demonstrated a biphasic pattern, characterized by an initial burst release followed by sustained release over an extended period. The initial burst may be attributed to the release of drug adsorbed on the nanoparticle surface, while the sustained phase is governed by diffusion through the polymeric matrix. Kinetic modelling indicated that the release followed the Higuchi model, suggesting a diffusion-controlled mechanism. This type of release profile is particularly beneficial in cancer therapy, where an initial therapeutic concentration is

required for rapid action, followed by sustained drug availability to maintain cytotoxic effects over time. Such controlled release behaviour reduces dosing frequency and minimizes systemic toxicity.

4.5 Structural Transformation and Its Impact on Drug Performance

DSC and XRD analyses revealed a reduction in crystallinity of paclitaxel within the nanocarrier system, indicating partial amorphization or molecular dispersion. This transformation is significant, as amorphous forms of drugs typically exhibit higher solubility and dissolution rates compared to their crystalline counterparts. The enhanced solubility of paclitaxel within the nanoparticle matrix likely contributed to improved bioavailability and cytotoxic activity. This finding aligns with previous studies reporting that nanoparticle encapsulation can overcome solubility limitations of hydrophobic drugs.

4.6 Enhanced Cytotoxicity and Therapeutic Efficiency

The cytotoxicity study demonstrated a substantial improvement in anticancer activity of the nanocarrier formulation compared to free drug. The approximately 2.3-fold reduction in IC_{50} value indicates enhanced therapeutic efficiency, which can be attributed to multiple factors, including improved cellular uptake, sustained drug release, and increased solubility. The use of MDA-MB-231 further strengthens the clinical relevance of the findings, as this cell line represents an aggressive and treatment-resistant subtype of breast cancer. The improved efficacy observed in this model suggests potential applicability of the formulation in overcoming chemoresistance associated with triple-negative breast cancer.

4.7 Stability and Practical Implications

Stability studies confirmed that the optimized formulation maintained its physicochemical properties over time, with minimal variation in particle size, zeta potential, and drug content. This indicates that the developed nanocarrier system possesses adequate stability for potential storage and transportation. From a translational perspective, the use of simple preparation techniques such as ionic gelation, combined with green synthesis principles, enhances the feasibility of large-scale production. The absence of complex instrumentation or hazardous reagents further supports industrial applicability.

4.8 Comparison with Existing Nanocarrier Systems

When compared with conventional nanoparticle systems reported in literature, the present formulation demonstrated several advantages, including:

- Use of eco-friendly synthesis approach
- Enhanced surface functionality via chitosan coating
- Improved cytotoxic performance
- Sustained drug release profile

Many previously reported systems rely on synthetic polymers or toxic solvents, which may limit their clinical translation. The current study addresses these limitations by integrating green chemistry with nanotechnology.

5. Conclusion

The present study successfully demonstrated the development of a green-synthesized, chitosan-coated nanocarrier system for the efficient delivery of Paclitaxel aimed at improving therapeutic outcomes in Triple-negative breast cancer. The integration of green synthesis principles with nanotechnology provided a sustainable and biocompatible approach for nanoparticle fabrication, avoiding the use of toxic solvents and synthetic stabilizers. The optimized nanocarrier formulation exhibited desirable physicochemical properties, including nanoscale particle size, narrow size distribution, high positive zeta potential, and excellent drug entrapment efficiency. Structural characterization confirmed successful encapsulation of paclitaxel within the polymeric matrix and partial amorphization of the drug, which contributed to enhanced solubility and bioavailability. The biphasic drug release profile, governed by diffusion-controlled kinetics, ensured an initial therapeutic burst followed by sustained release, which is highly advantageous in cancer therapy. Biological evaluation revealed significantly improved cytotoxicity of the nanocarrier formulation compared to free drug, as evidenced by a marked reduction in IC_{50} values in MDA-MB-231 cells. Enhanced cellular uptake further supported the role of chitosan coating in facilitating efficient internalization of nanoparticles through electrostatic interactions. Stability studies confirmed that the formulation remained stable under accelerated and long-term storage conditions, highlighting its potential for practical application. Overall, the developed nanocarrier system offers a promising platform for targeted and efficient delivery of paclitaxel, with potential to overcome limitations associated with conventional chemotherapy. Future studies focusing on in vivo evaluation and clinical translation are warranted to fully establish its therapeutic potential.

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