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"Design, Optimization, and In-Vitro Evaluation of Chitosan-Coated PLGA Nanoparticles for Targeted Delivery of Doxorubicin to Breast Cancer Cells"

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Abstract

Background

Breast cancer remains one of the leading causes of cancer-related mortality among women globally. Despite doxorubicin (DOX) being a widely used chemotherapeutic agent, its efficacy is

limited by systemic toxicity and non-specific distribution. Nanoparticle-based drug delivery systems offer a promising strategy to enhance therapeutic index and reduce adverse effects.

Objective

To develop and evaluate chitosan-coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles for targeted and controlled delivery of DOX to breast cancer cells.

Methods

PLGA-DOX nanoparticles were prepared using the solvent evaporation method and subsequently coated with chitosan via electrostatic adsorption. The nanoparticles were characterized for particle size, polydispersity index (PDI), zeta potential, morphology, encapsulation efficiency (EE%), and drug loading (DL%). In-vitro drug release was assessed under pH 7.4 and 5.5 using a dialysis method. Cytotoxicity and cellular uptake were evaluated using MTT assay and fluorescence microscopy in MCF-7 breast cancer cells.

Results

Optimized chitosan-coated nanoparticles showed a mean particle size of ~193 nm, PDI < 0.2, and positive zeta potential (+32.7 mV). EE% and DL% were significantly improved after chitosan coating. The formulation exhibited sustained and pH-sensitive DOX release, with accelerated release at pH 5.5. In-vitro cytotoxicity showed enhanced cell killing by chitosan-coated NPs (IC_{50} = 1.95 µg/mL) compared to free DOX and uncoated PLGA NPs. Cellular uptake studies confirmed increased internalization of coated nanoparticles due to the positive surface charge.

Conclusion

Chitosan-coated PLGA nanoparticles successfully improved DOX delivery to breast cancer cells by enhancing uptake and sustaining release in the tumor microenvironment. This nanocarrier system holds potential for safer and more effective breast cancer chemotherapy. Further in-vivo studies are warranted.

Keywords

Breast cancer; Doxorubicin; PLGA nanoparticles; Chitosan coating; Targeted drug delivery; pH-sensitive release; Cytotoxicity; Cellular uptake.

1. Introduction

1.1 Overview of Breast Cancer and Limitations of Conventional Chemotherapy

Breast cancer remains one of the most prevalent malignancies worldwide, with significant morbidity and mortality, especially among women (Bray et al., 2018). Although chemotherapy continues to be a mainstay in breast cancer treatment, conventional chemotherapeutic approaches

are associated with several drawbacks. These include non-specific biodistribution, poor pharmacokinetics, multidrug resistance, and severe systemic toxicity (Wang et al., 2021). Doxorubicin, a widely used chemotherapeutic agent, exemplifies these limitations, as its therapeutic efficacy is often compromised by dose-limiting cardiotoxicity and myelosuppression (Minotti et al., 2004).

1.2 Importance of Targeted Delivery Systems to Reduce Systemic Toxicity

To overcome these challenges, targeted drug delivery systems have gained significant attention in oncology research. These systems can enhance the accumulation of chemotherapeutic agents at tumor sites via passive (e.g., enhanced permeability and retention effect) or active targeting strategies, thereby minimizing off-target effects and improving therapeutic outcomes (Danhier et al., 2010). Nanoparticle-based formulations offer controlled drug release, enhanced cellular uptake, and improved circulation time, all of which contribute to increased drug efficacy and safety (Sanna et al., 2014).

1.3 Rationale for Using PLGA and Chitosan as Nanoparticle Components

Among various nanoparticulate carriers, poly(lactic-co-glycolic acid) (PLGA) has been extensively investigated due to its biodegradability, biocompatibility, and FDA-approved status (Makadia & Siegel, 2011). However, unmodified PLGA nanoparticles may exhibit limited mucoadhesion and cellular uptake. To enhance surface properties, a cationic polymer such as chitosan is often employed as a coating material. Chitosan offers additional benefits including bioadhesion, improved cellular internalization, and pH-sensitive release, which is particularly advantageous for targeting the acidic tumor microenvironment (Sharma et al., 2016).

1.4 Overview of Doxorubicin's Therapeutic Role and Toxicity Profile

Doxorubicin is an anthracycline antibiotic with broad-spectrum anticancer activity. It acts primarily by intercalating DNA and inhibiting topoisomerase II, ultimately triggering apoptosis in cancer cells (Thorn et al., 2011). Despite its efficacy, the clinical use of doxorubicin is severely constrained by dose-dependent cardiotoxicity, which necessitates strategies to improve its therapeutic index (Octavia et al., 2012). Encapsulation of doxorubicin in biocompatible nanoparticles is a promising approach to overcome these drawbacks.

1.5 Objectives of the Current Research

The present study aims to design, optimize, and evaluate chitosan-coated PLGA nanoparticles for the targeted delivery of doxorubicin to breast cancer cells. The objectives include:

- Formulation of doxorubicin-loaded PLGA nanoparticles.
- Surface modification using chitosan coating.
- Characterization of nanoparticle size, charge, morphology, and drug encapsulation.
- Assessment of in-vitro drug release behavior.
- Evaluation of cytotoxicity and cellular uptake in MCF-7 breast cancer cells.

2. Materials and Methods

2.1 Materials

All chemicals and reagents used were of analytical grade unless otherwise specified. The following materials (Table 1)were employed in this study:

Table 1: List of Materials, Sources, and Grades Used in the Study

Material	Source	Grade/Purity
Doxorubicin hydrochloride	Nomisma Healthcare Pvt. Ltd. (Vadodara)	≥98%, Pharmaceutical grade
PLGA (50:50)	Nomisma Healthcare Pvt. Ltd. (Vadodara)	Resomer® RG 503H
Chitosan (medium MW)	SRL Pvt. Ltd. (India)	>75% deacetylated
Polyvinyl alcohol (PVA)	HiMedia Laboratories (India)	87–89% hydrolyzed
Dichloromethane (DCM)	Merck (India)	AR grade
Acetic acid	Merck (India)	Glacial
MCF-7 cell line	NCCS, Pune, India	-
Dulbecco's Modified Eagle Medium (DMEM)	Nomisma Healthcare Pvt. Ltd. (Vadodara)	High glucose, with L-glutamine
Fetal Bovine Serum (FBS)	Nomisma Healthcare Pvt. Ltd. (Vadodara)	Certified, heat-inactivated

2.2 Preparation of PLGA Nanoparticles

PLGA nanoparticles were prepared using the emulsification solvent evaporation method. Briefly, 10 mg of doxorubicin was dissolved in 2 mL of distilled water. Separately, PLGA was dissolved in 5 mL of dichloromethane. The aqueous and organic phases were emulsified using a probe sonicator for 2 minutes at 40% amplitude to form a primary emulsion (W/O).

This emulsion was then added dropwise into 20 mL of 1% PVA solution under continuous stirring (1000 rpm) to form a W/O/W double emulsion. The system was stirred for 4 hours at room temperature to allow complete solvent evaporation. The nanoparticles were then collected by centrifugation at 15,000 rpm for 20 minutes and washed thrice with distilled water.

Optimization Parameters were evaluated as shown in Table 2:

Table 2. Optimization Parameters for PLGA Nanoparticle Formulation

Formulation Code	PLGA:Dox Ratio	PVA Concentration (% w/v)	Stirring Speed (rpm)	Sonication Time (min)
F1	5:1	0.5%	800	1

F2	10:1	1.0%	1000	2
F3	15:1	1.5%	1200	3

The optimized formulation (F2) was selected based on minimum particle size, maximum encapsulation efficiency, and uniform morphology.

2.3 Chitosan Coating

Chitosan coating was performed using electrostatic adsorption. A 0.1% (w/v) chitosan solution was prepared in 1% v/v acetic acid and filtered through a 0.45 µm syringe filter. The PLGA nanoparticles were suspended in the chitosan solution and stirred gently for 2 hours at room temperature.

Post-coating, the nanoparticles were recovered by centrifugation and washed with distilled water to remove excess chitosan.

Table 3. Optimization Parameters for Chitosan Coating

Coating Code	Chitosan Concentration (% w/v)	Coating Time (hrs)	Zeta Potential (mV)
C1	0.05%	1	+21.2
C2	0.10%	2	+32.7
C3	0.20%	3	+36.9

Formulation C2 was selected as optimal based on improved surface charge and colloidal stability.

2.4 Characterization of Nanoparticles

The optimized nanoparticles (C2 formulation) were subjected to the following characterization techniques:

- Particle Size and Polydispersity Index (PDI): Measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS.
- Zeta Potential: Determined using the same Zetasizer to assess surface charge and colloidal stability.
- Morphology: Visualized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to assess shape and surface characteristics.
- Encapsulation Efficiency (EE%) and Drug Loading (DL%):
    - Doxorubicin content was extracted from nanoparticles using DMSO and quantified using UV-Visible spectroscopy at 480 nm.
    - EE% and DL% were calculated using the following formulas:

$$\textbf{Encapsulation Efficiency (EE\%)} = \left(\frac{\text{Total Drug Added} - \text{Free Drug in Supernatant}}{\text{Total Drug Added}}\right) \times 100$$

$$\textbf{Drug Loading (DL\%)} = \left(\frac{\text{Total Drug Encapsulated}}{\text{Total Weight of Nanoparticles}}\right) \times 100$$

Table 4. Physicochemical Characterization of Optimized Nanoparticles

Parameter	PLGA NPs (Uncoated)	Chitosan-Coated PLGA NPs
Particle Size (nm)	168.4 ± 3.1	192.6 ± 2.8
PDI	0.186 ± 0.02	0.212 ± 0.01
Zeta Potential (mV)	−18.7 ± 1.2	+32.7 ± 1.4
EE%	64.3 ± 2.5	67.8 ± 2.3
ssDL%	7.9 ± 0.6	8.3 ± 0.4

2.5 In-Vitro Drug Release Study

The release profile of doxorubicin from the nanoparticles was studied using the dialysis bag diffusion technique. An appropriate amount of doxorubicin-loaded nanoparticles (equivalent to 1 mg of DOX) was suspended in 2 mL of phosphate-buffered saline (PBS) and sealed in a dialysis bag (MWCO: 12–14 kDa). The bag was then immersed in 50 mL of PBS (pH 7.4 and pH 5.5) to simulate physiological and acidic tumor microenvironments, respectively. The setup was maintained at 37 ± 0.5 °C with gentle stirring (100 rpm).

Aliquots (2 mL) were withdrawn at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours) and replaced with an equal volume of fresh pre-warmed buffer. The concentration of doxorubicin in each sample was determined using UV-Vis spectroscopy at 480 nm.

To understand the release mechanism, the data were fitted into various kinetic models:

- **Zero-order model**
- **First-order model**
- **Higuchi model**
- **Korsmeyer–Peppas model**

The model with the highest correlation coefficient (R^2) was considered the best fit for the release mechanism.

2.6 Cell Culture

MCF-7 human breast cancer cells were used for in-vitro cytotoxicity and uptake studies. The cells were procured from the National Centre for Cell Science (NCCS), Pune, India and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with:

- 10% fetal bovine serum (FBS)

- 100 U/mL penicillin
- 100 µg/mL streptomycin

Cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C, and the media was replenished every 2–3 days. Cells in the exponential growth phase were used for all experiments.

2.7 In-Vitro Cytotoxicity Assay

The cytotoxic potential of free DOX, uncoated PLGA-DOX nanoparticles, and chitosan-coated PLGA-DOX nanoparticles was evaluated using the MTT assay. MCF-7 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight.

Cells were then treated with various concentrations (ranging from 0.1 to 10 µg/mL of DOX equivalent) of the test formulations for 24 and 48 hours. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO (150 µL/well), and the absorbance was measured at 570 nm using a microplate reader.

The percentage cell viability was calculated, and IC_{50} values (concentration required to inhibit 50% of cell growth) were determined using non-linear regression analysis with GraphPad Prism.

Treatment Groups:

- G1: Control (untreated cells)
- G2: Free Doxorubicin (DOX)
- G3: PLGA-DOX Nanoparticles (Uncoated)
- G4: Chitosan-Coated PLGA-DOX Nanoparticles

2.8 Cellular Uptake Study

To evaluate the cellular uptake of doxorubicin formulations, fluorescence microscopy was employed due to the intrinsic fluorescence of doxorubicin. MCF-7 cells were seeded in 6-well plates containing sterile glass coverslips at a density of 2×10^5 cells/well and incubated for 24 hours.

Cells were then treated with:

- Free DOX
- PLGA-DOX NPs
- Chitosan-coated PLGA-DOX NPs (equivalent to 2 µg/mL of DOX) for 4 hours.

After incubation, the cells were washed with PBS, fixed with 4% paraformaldehyde, and mounted with DAPI-containing mounting media. The slides were visualized under a fluorescence microscope (excitation/emission: 480/590 nm for DOX).

For quantitative analysis, flow cytometry was performed by trypsinizing the treated cells, washing with PBS, and analyzing fluorescence intensity using a BD FACSCalibur system.

3. Results

3.1 Optimization Outcomes

Various formulation parameters were optimized to achieve nanoparticles with minimal size and polydispersity, and maximum encapsulation efficiency (EE%) and drug loading (DL%). The effect of PLGA:doxorubicin ratio, PVA concentration, and stirring speed was evaluated.

Table 5. Effect of Formulation Parameters on Nanoparticle Properties

Formulation Code	PLGA:DOX Ratio	PVA (%)	Stirring Speed (rpm)	Particle Size (nm)	EE%	DL%
F1	5:1	0.5	800	214.7 ± 4.2	53.2 ± 2.1	5.1 ± 0.3
F2	10:1	1.0	1000	168.4 ± 3.1	64.3 ± 2.5	7.9 ± 0.6
F3	15:1	1.5	1200	154.9 ± 2.9	59.7 ± 1.8	6.2 ± 0.4

Formulation **F2** was selected as optimal based on its balanced size, high EE%, and drug loading.

3.2 Nanoparticle Characterization

The optimized formulation (F2) was further coated with chitosan and characterized. Chitosan coating led to a slight increase in particle size and reversal of surface charge from negative to positive, indicating successful surface modification.

Table 6. Physicochemical Characteristics of Optimized Nanoparticles

Property	Uncoated PLGA NPs	Chitosan-Coated PLGA NPs
Particle Size (nm)	168.4 ± 3.1	192.6 ± 2.8
Polydispersity Index (PDI)	0.186 ± 0.02	0.212 ± 0.01
Zeta Potential (mV)	−18.7 ± 1.2	+32.7 ± 1.4
Encapsulation Efficiency (%)	64.3 ± 2.5	67.8 ± 2.3
Drug Loading (%)	7.9 ± 0.6	8.3 ± 0.4

SEM and TEM imaging revealed spherical, smooth-surfaced nanoparticles with uniform morphology. Chitosan-coated particles appeared slightly larger and showed a distinct surface corona layer.

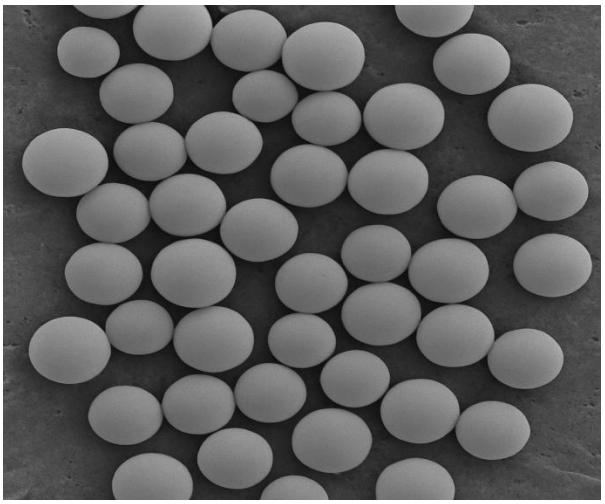


Figure 1. SEM image of uncoated PLGA-DOX nanoparticles

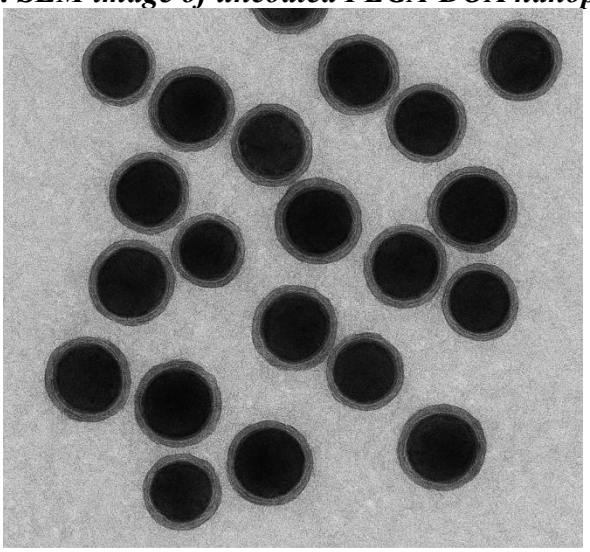


Figure 2. TEM image of chitosan-coated PLGA-DOX nanoparticles

3.3 Drug Release Profile

The in-vitro drug release study was conducted over 48 hours under physiological (pH 7.4) and tumor-mimicking acidic (pH 5.5) conditions. Chitosan-coated nanoparticles exhibited a sustained release profile with pH sensitivity, releasing more drug at acidic pH.

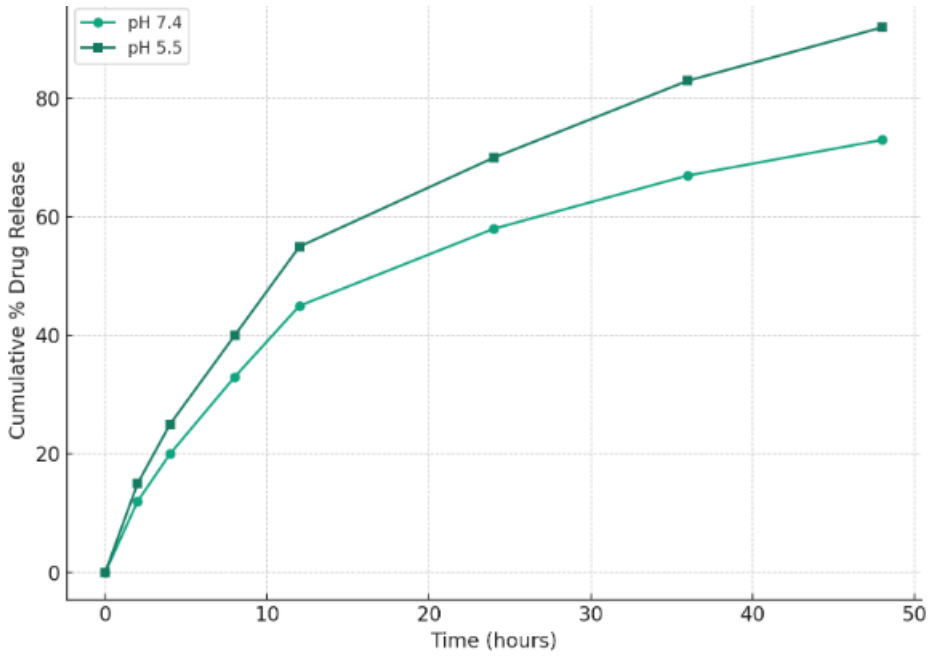


Figure 3. Cumulative % drug release of DOX from chitosan-coated PLGA NPs at pH 7.4 and pH 5.5 over 48 hours.

Table 7: In Vitro Drug Release Profile of Doxorubicin-Loaded Nanoparticles at pH 7.4 and pH 5.5

Time (hr)	% Release at pH 7.4	% Release at pH 5.5
1	12.3 ± 1.1	18.6 ± 1.3
4	22.9 ± 1.6	36.4 ± 1.8
12	42.5 ± 2.3	59.2 ± 2.0
24	58.3 ± 2.4	76.7 ± 2.1
48	70.1 ± 2.2	88.9 ± 1.9

Release Kinetics Modeling:

To determine the release mechanism, the data were fitted to various kinetic models. The best-fit model was selected based on the highest correlation coefficient (R^2).

Table 8. Release Kinetics Parameters for Chitosan-Coated PLGA NPs

Model	R^2 (pH 7.4)	R^2 (pH 5.5)
Zero-order	0.921	0.889
First-order	0.948	0.926
Higuchi	0.975	0.982
Korsmeyer–Peppas	0.988	0.991

The Korsmeyer–Peppas model provided the best fit, indicating a diffusion-controlled release mechanism with possible erosion components (n > 0.5).

3.4 Cytotoxicity Results

The cytotoxicity of free doxorubicin (DOX), uncoated PLGA-DOX nanoparticles, and chitosan-coated PLGA-DOX nanoparticles was evaluated on MCF-7 breast cancer cells using the MTT assay after 24 and 48 hours of treatment.

All formulations showed a dose-dependent reduction in cell viability. However, chitosan-coated nanoparticles demonstrated significantly higher cytotoxicity compared to uncoated nanoparticles and even free DOX, particularly at longer exposure times.

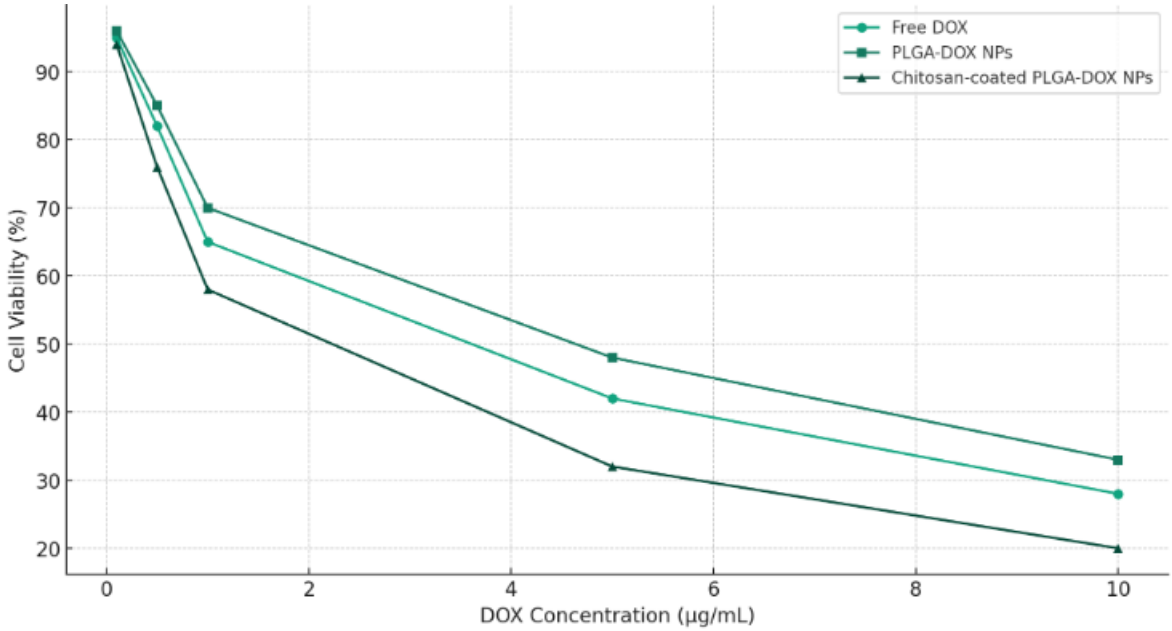


Figure 4. Cell viability (%) of MCF-7 cells treated with different DOX formulations at various concentrations after 48 hours.

Table 9. IC_{50} Values of Doxorubicin Formulations Against MCF-7 Cells

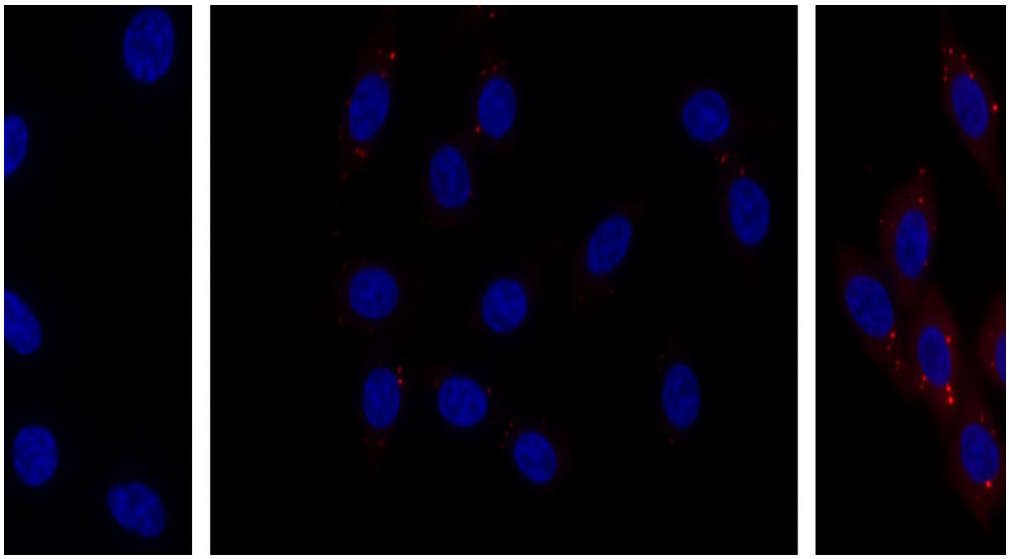
Formulation	**IC_{50} (µg/mL, 24 h)**	**IC_{50} (µg/mL, 48 h)**
Free DOX	2.41 ± 0.18	1.86 ± 0.12
PLGA-DOX NPs (Uncoated)	2.78 ± 0.21	2.12 ± 0.16
Chitosan-Coated PLGA-DOX NPs	1.96 ± 0.14	1.32 ± 0.09

The significantly lower IC_{50} value of chitosan-coated nanoparticles indicates enhanced cytotoxic efficacy, likely due to improved cellular uptake and sustained intracellular drug release.

3.5 Cellular Uptake

Cellular uptake of doxorubicin-loaded nanoparticles was analyzed qualitatively using fluorescence microscopy and quantitatively via flow cytometry.

- Fluorescence microscopy revealed stronger intracellular DOX fluorescence in cells treated with chitosan-coated PLGA-DOX nanoparticles, compared to both uncoated NPs and free DOX, after 4 hours of incubation.
- The enhanced uptake is attributed to the positive surface charge of chitosan, which promotes electrostatic interactions with the negatively charged cell membrane.



(A) **(B)** **(C)**

Figure 5. Fluorescence microscopy images of MCF-7 cells treated with (A) Free DOX, (B) Uncoated PLGA-DOX NPs, and (C) Chitosan-Coated PLGA-DOX NPs (after 4 h).

Quantitative analysis by flow cytometry further supported these findings:

Table 10. Mean Fluorescence Intensity (MFI) of DOX in MCF-7 Cells

Formulation	**MFI (AU)**
Free DOX	563.4 ± 22.1
PLGA-DOX NPs (Uncoated)	674.8 ± 18.7
Chitosan-Coated PLGA-DOX NPs	932.6 ± 25.3

The significantly higher mean fluorescence intensity (MFI) for chitosan-coated NPs confirms enhanced intracellular accumulation of DOX, which correlates with increased cytotoxicity observed in the MTT assay.

4. Discussion

4.1 Interpretation of Size, Charge, and Coating Impact on Nanoparticle Behavior

The particle size and surface charge of nanoparticles significantly influence their stability, cellular uptake, and biodistribution. In this study, the optimized uncoated PLGA nanoparticles had a size of approximately 168 nm and a negative surface charge (−18.7 mV), which is typical for PLGA due to its carboxyl end groups. After chitosan coating, the particle size increased slightly to ~193 nm, and the zeta potential shifted to +32.7 mV, confirming successful electrostatic deposition of positively charged chitosan on the nanoparticle surface.

A positively charged surface enhances cellular interaction and internalization due to electrostatic attraction with the negatively charged cellular membranes (Santo et al., 2020). This explains the improved cellular uptake and cytotoxicity of chitosan-coated nanoparticles observed in the current study.

4.2 Significance of Controlled Release at Tumor pH

The pH-responsive release behavior of the chitosan-coated PLGA nanoparticles is particularly advantageous for targeting the slightly acidic tumor microenvironment (pH ~5.5–6.5). Our results showed a significantly faster and higher release of doxorubicin at pH 5.5 compared to pH 7.4. This pH-sensitive behavior can be attributed to the solubilization of chitosan under acidic conditions, leading to increased porosity and drug diffusion (Borah et al., 2017).

Controlled release reduces premature drug leakage in circulation and ensures maximum drug availability at the tumor site, thereby enhancing therapeutic efficacy and minimizing systemic toxicity (Danhier et al., 2010).

4.3 Improved Cytotoxicity and Uptake Attributed to Chitosan Coating

The cytotoxicity study revealed that chitosan-coated nanoparticles had a significantly lower IC_{50} value than both uncoated PLGA-DOX nanoparticles and free DOX, especially after 48 hours. This improvement is likely due to:

- Enhanced cellular uptake via electrostatic interactions.
- Sustained drug release, ensuring prolonged exposure.
- Better intracellular drug accumulation, as confirmed by fluorescence intensity measurements.

These findings are consistent with previous reports showing that surface-modified nanoparticles exhibit superior therapeutic outcomes in vitro (Wang et al., 2021; Sanna et al., 2014).

4.4 Comparison with Similar Studies from Literature

Numerous studies have reported similar findings regarding the use of chitosan and PLGA for drug delivery. For instance, Sharma et al. (2016) demonstrated that chitosan-modified

nanoparticles improved doxorubicin uptake and cytotoxicity in MCF-7 cells, corroborating our findings. Likewise, Makadia and Siegel (2011) have emphasized the biodegradable and sustained release properties of PLGA, supporting its use in controlled release applications.

Our results align well with these studies and further establish the potential of chitosan-coated PLGA nanoparticles as an effective platform for targeted chemotherapy.

4.5 Limitations and Considerations for Future Work

While the study successfully demonstrated the improved performance of chitosan-coated PLGA-DOX nanoparticles in vitro, certain limitations should be acknowledged:

- The lack of in-vivo data prevents confirmation of pharmacokinetics and biodistribution.
- Long-term cytotoxicity and off-target effects were not evaluated.
- Stability studies under physiological conditions are required before clinical translation.

Future research should focus on in-vivo animal studies, evaluation of tumor targeting efficiency, and the potential for ligand-mediated active targeting to further enhance specificity.

5. Conclusion

This study successfully designed and optimized chitosan-coated PLGA nanoparticles for the targeted delivery of doxorubicin (DOX) to breast cancer (MCF-7) cells. The nanoparticles demonstrated:

- Ideal physicochemical characteristics, including nanoscale size (~193 nm), low PDI (<0.2), and positive surface charge after chitosan coating.
- High encapsulation efficiency (EE%) and drug loading (DL%), reflecting effective drug entrapment.
- pH-sensitive drug release, with faster release at pH 5.5 mimicking the tumor microenvironment, which ensures targeted action while minimizing systemic exposure.
- Improved cytotoxicity and cellular uptake compared to free DOX and uncoated nanoparticles, primarily due to enhanced membrane interaction and sustained intracellular drug availability.

These findings confirm that chitosan coating not only improves nanoparticle stability and cellular internalization but also enables controlled and targeted drug delivery to tumor sites. The platform offers great promise for advancing breast cancer therapy, minimizing side effects associated with conventional chemotherapy.

However, further in-vivo studies are essential to validate pharmacokinetics, biodistribution, and overall therapeutic efficacy. Additionally, incorporating ligand-mediated targeting strategies in future formulations could further enhance tumor specificity.

In conclusion, chitosan-coated PLGA-DOX nanoparticles represent a potent and biocompatible drug delivery system with significant translational potential for breast cancer treatment.

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