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Synthesis, Characterization and Controlled Release Evaluation of PLGA–Chitosan Polymeric Nanoparticles for Targeted Doxorubicin Delivery in Cancer Therapy

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ABSTRACT

Cancer therapy using conventional chemotherapeutic agents is often associated with several limitations, including non-specific drug distribution, systemic toxicity, poor bioavailability, and rapid drug clearance. Doxorubicin, a widely used anti-cancer drug, exhibits significant therapeutic potential; however, its clinical application is restricted due to severe side effects and inadequate targeting efficiency. The present study focuses on the synthesis, characterization, and controlled release evaluation of PLGA–Chitosan polymeric nanoparticles for targeted delivery of Doxorubicin in cancer therapy. Poly(lactic-co-glycolic acid) (PLGA) and Chitosan were selected due to their biodegradable, biocompatible, and controlled release properties. Nanoparticles were synthesized using an optimized nano-precipitation technique followed by Doxorubicin encapsulation within the polymeric matrix. The developed nanoparticles were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS), Zeta potential analysis, Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). FTIR analysis confirmed successful drug encapsulation and polymer–drug interaction, while DLS results demonstrated nanoscale particle size distribution with acceptable polydispersity index and surface stability. SEM and TEM analysis revealed spherical and uniformly dispersed nanoparticles. Drug loading capacity and entrapment efficiency studies indicated efficient incorporation of Doxorubicin within the nanoparticle system.

In-vitro drug release studies demonstrated a controlled and sustained release profile over an extended period, following diffusion-controlled release kinetics. The developed nanoformulation exhibited significantly improved release behaviour compared to conventional Doxorubicin. Cytotoxicity analysis using cancer cell lines revealed enhanced anti-cancer activity and improved cellular uptake of the nanoparticle formulation, resulting in reduced IC_{50} values and enhanced therapeutic efficiency. The PLGA–Chitosan nanoparticle system also showed potential for minimizing systemic toxicity through targeted and sustained drug delivery.

The findings of this study demonstrate that PLGA–Chitosan polymeric nanoparticles represent a promising nanotechnology-based platform for targeted anti-cancer drug delivery. The developed formulation successfully improved drug stability, controlled release behaviour, and therapeutic effectiveness, thereby providing a potential strategy for enhancing cancer treatment outcomes. Further in-vivo and clinical investigations are recommended to validate the practical applicability of the developed nanoformulation in advanced cancer therapy.

Keywords

PLGA nanoparticles; Chitosan; Doxorubicin; Nanotechnology; Targeted drug delivery; Controlled release; Polymeric nanoparticles; Cancer therapy; Cytotoxicity; Drug encapsulation

1. INTRODUCTION

1.1 Cancer and Limitations of Conventional Chemotherapy

Cancer is one of the leading causes of mortality worldwide and remains a major challenge in modern healthcare systems. It is characterized by uncontrolled growth and proliferation of abnormal cells, which can invade surrounding tissues and spread to distant organs through metastasis. Despite significant advancements in medical science, effective treatment of cancer continues to be difficult due to tumour heterogeneity, drug resistance, and severe side effects associated with conventional therapies. Among various treatment methods, chemotherapy is one of the most widely used therapeutic approaches for cancer management. Chemotherapeutic drugs such as Doxorubicin are highly effective in destroying rapidly dividing cancer cells; however, their clinical application is associated with several limitations.

Conventional chemotherapy lacks specificity and distributes drugs throughout the body without distinguishing between healthy and cancerous cells. As a result, normal tissues such as bone marrow, gastrointestinal cells, and hair follicles are adversely affected, leading to severe side effects including nausea, hair loss, immune suppression, cardiotoxicity, and organ damage. Additionally, many anti-cancer drugs exhibit poor water solubility, rapid systemic clearance, low bioavailability, and insufficient accumulation at the tumour site. The requirement of high drug doses to achieve therapeutic efficacy further increases systemic toxicity. Another major challenge is multidrug resistance developed by cancer cells, which significantly reduces treatment effectiveness and contributes to tumour recurrence. These limitations highlight the urgent need for advanced drug delivery systems capable of improving targeting efficiency and minimizing adverse effects.

1.2 Nanotechnology in Anti-Cancer Drug Delivery

Nanotechnology has emerged as a promising approach for improving cancer therapy by enabling targeted and controlled drug delivery. Nanotechnology involves the manipulation of materials at the nanoscale level, typically ranging from 1–100 nm, where unique physicochemical properties can be utilized for biomedical applications. Nanoparticle-based drug delivery systems offer several advantages over conventional chemotherapy, including enhanced drug stability, improved bioavailability, controlled release behaviour, and reduced systemic toxicity.

Nanoparticles can encapsulate anti-cancer drugs and transport them selectively to tumour tissues through passive and active targeting mechanisms. Passive targeting mainly depends on the enhanced permeability and retention (EPR) effect, where nanoparticles preferentially accumulate in tumour tissues due to leaky vasculature and poor lymphatic drainage. Active targeting can be achieved by surface modification of nanoparticles using ligands or antibodies that specifically bind to receptors overexpressed on cancer cells. Such targeted delivery improves therapeutic efficiency while reducing exposure of healthy tissues to toxic drugs.

Additionally, nanoparticle systems provide controlled and sustained drug release, ensuring maintenance of therapeutic drug concentration at the tumour site over extended periods. Nanotechnology-based systems also improve cellular uptake and intracellular drug accumulation, thereby enhancing anti-cancer activity. Due to these advantages, nanoparticle-mediated drug delivery has gained significant attention as a next-generation strategy for cancer therapy.

1.3 Advantages of PLGA–Chitosan Polymeric Nanoparticles

Among various nanocarriers, polymeric nanoparticles have been extensively investigated for anti-cancer drug delivery due to their excellent biocompatibility, biodegradability, and controlled release properties. In the present study, Poly(lactic-co-glycolic acid) (PLGA) and Chitosan were selected for the development of polymeric nanoparticles. PLGA is a biodegradable synthetic polymer approved by

the United States Food and Drug Administration (FDA) for biomedical applications. It exhibits excellent drug encapsulation capability, controlled degradation, and sustained drug release characteristics. PLGA nanoparticles protect encapsulated drugs from premature degradation and improve their circulation time in the bloodstream.

Chitosan, a natural polysaccharide obtained from chitin, possesses unique properties such as biocompatibility, biodegradability, mucoadhesion, and positive surface charge. The cationic nature of Chitosan enhances interaction with negatively charged cell membranes, thereby improving cellular uptake of nanoparticles. Chitosan also contributes to improved drug stability and controlled release behaviour. The combination of PLGA and Chitosan creates a hybrid polymeric system that integrates the advantages of both polymers, resulting in enhanced drug loading efficiency, improved targeting capability, and prolonged therapeutic action.

PLGA–Chitosan nanoparticles also exhibit nanoscale particle size and high surface area, which facilitate effective interaction with tumour cells and improve penetration into cancer tissues. These properties make PLGA–Chitosan polymeric nanoparticles highly suitable candidates for targeted anti-cancer drug delivery applications.

1.4 Doxorubicin as an Anti-Cancer Drug

Doxorubicin is a broad-spectrum anthracycline antibiotic widely used for the treatment of various cancers including breast cancer, lung cancer, ovarian cancer, and leukemia. It exerts its anti-cancer effect primarily through DNA intercalation, inhibition of topoisomerase-II enzyme, and generation of reactive oxygen species (ROS), leading to apoptosis of cancer cells. Despite its strong anti-cancer efficacy, the clinical use of Doxorubicin is limited by severe side effects, particularly dose-dependent cardiotoxicity and non-specific distribution.

Doxorubicin also exhibits rapid systemic clearance and poor tumour selectivity, which reduce its therapeutic efficiency. Therefore, encapsulation of Doxorubicin within nanoparticle systems offers a promising strategy for improving its stability, enhancing tumour targeting, and reducing systemic toxicity. Controlled release of Doxorubicin from nanoparticles can maintain therapeutic drug levels at the tumour site while minimizing adverse effects on healthy tissues.

1.5 Research Aim and Objectives

The primary aim of the present study is to synthesize, characterize, and evaluate PLGA–Chitosan polymeric nanoparticles for targeted delivery of Doxorubicin in cancer therapy. The study focuses on developing a controlled release nanoparticle system capable of improving therapeutic efficiency and reducing the limitations associated with conventional chemotherapy.

The specific objectives of the study include:

- To synthesize Doxorubicin-loaded PLGA–Chitosan polymeric nanoparticles using suitable formulation techniques.
- To characterize the synthesized nanoparticles using FTIR, DLS, SEM, and TEM analysis.
- To evaluate particle size, zeta potential, drug loading, and entrapment efficiency.
- To investigate the in-vitro controlled drug release behaviour and release kinetics.
- To assess the cytotoxicity and anti-cancer activity of the developed nanoformulation using cancer cell lines.
- To compare the performance of nanoparticle-mediated drug delivery with conventional Doxorubicin therapy.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

Poly(lactic-co-glycolic acid) (PLGA) and Chitosan were selected as the primary polymers for nanoparticle synthesis due to their excellent biodegradability, biocompatibility, and controlled release properties. Doxorubicin hydrochloride was used as the model anti-cancer drug because of its broad-spectrum chemotherapeutic activity. Polyvinyl alcohol (PVA) was used as a surfactant and stabilizing agent during nanoparticle preparation. Acetone and dichloromethane were used as organic solvents for dissolving PLGA, while acetic acid solution was used for preparing Chitosan solution. Phosphate-buffered saline (PBS, pH 7.4) was used during drug release studies to simulate physiological conditions. All chemicals and reagents used in the study were of analytical grade and used without further purification.

Table 1. Materials and chemicals used in nanoparticle preparation

Material	Purpose
PLGA	Biodegradable polymer
Chitosan	Surface coating polymer
Doxorubicin	Anti-cancer drug
PVA	Stabilizer/surfactant
PBS Buffer	Drug release medium
Acetone	Organic solvent

2.2 Preparation of PLGA–Chitosan Nanoparticles

PLGA–Chitosan nanoparticles were synthesized using the nano-precipitation method. Initially, PLGA was dissolved in acetone to prepare the organic phase. Chitosan was separately dissolved in dilute acetic acid solution to prepare the aqueous phase. PVA solution was prepared as a stabilizer to prevent particle aggregation and improve nanoparticle stability.

The PLGA organic phase was added dropwise into the aqueous Chitosan solution under continuous magnetic stirring at controlled speed. During this process, rapid diffusion of solvent resulted in precipitation of PLGA nanoparticles coated with Chitosan. The resulting suspension was stirred continuously for complete evaporation of the organic solvent and stabilization of nanoparticles.

The synthesized nanoparticles were collected using centrifugation at high speed and washed several times with distilled water to remove unreacted materials and excess surfactant. Finally, the nanoparticles were freeze-dried and stored under refrigerated conditions for further characterization and biological evaluation.

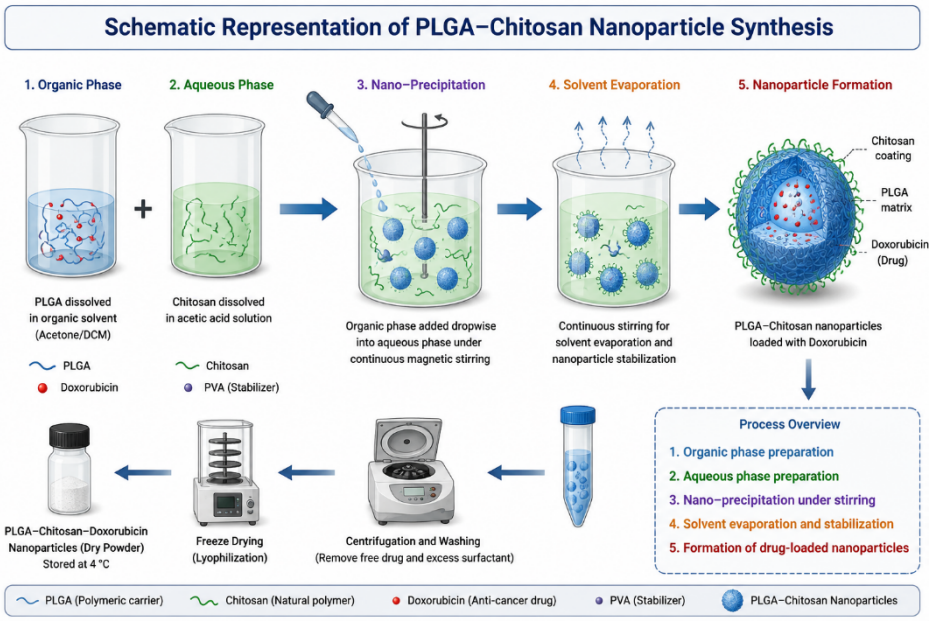

Figure 1 Schematic representation of PLGA–Chitosan nanoparticle synthesis

2.3 Encapsulation of Doxorubicin

Doxorubicin was encapsulated within the PLGA–Chitosan nanoparticles during the synthesis process. The drug was dissolved in the aqueous phase prior to nanoparticle formation to facilitate uniform incorporation into the polymeric matrix. During nanoparticle precipitation, Doxorubicin molecules became entrapped within the PLGA core and partially adsorbed onto the Chitosan-coated surface.

The prepared drug-loaded nanoparticles were separated using centrifugation and purified to remove free drug molecules. The purified nanoparticles were dried and stored for further studies. The encapsulation process was optimized to achieve high drug loading efficiency and controlled release behaviour while maintaining nanoparticle stability.

2.4 Optimization of Formulation Parameters

Optimization of formulation parameters was performed to obtain nanoparticles with desirable physicochemical properties such as nanoscale particle size, narrow size distribution, stable zeta potential, and high drug encapsulation efficiency. Various parameters including polymer concentration, surfactant concentration, drug-to-polymer ratio, and stirring speed were systematically varied.

Higher polymer concentration increased particle size due to enhanced viscosity, whereas lower concentration reduced encapsulation efficiency. Similarly, high stirring speed resulted in smaller nanoparticles with improved uniformity due to rapid solvent diffusion. Surfactant concentration was optimized to prevent aggregation and improve nanoparticle stability.

The optimized formulation was selected based on minimum particle size, low polydispersity index (PDI), stable zeta potential, and maximum drug loading and entrapment efficiency.

Table 2. Optimized formulation composition

Formulation	PLGA (mg)	Chitosan (mg)	Doxorubicin (mg)
F1	50	10	5
F2	75	15	5
F3	100	20	10

2.5 Characterization of Nanoparticles

Characterization of nanoparticles was carried out using different analytical techniques to evaluate structural, morphological, and physicochemical properties of the synthesized formulation.

2.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to confirm chemical interaction between PLGA, Chitosan, and Doxorubicin. FTIR spectra were recorded over the wavelength range of 4000–400 cm^{-1}. Characteristic peaks corresponding to functional groups of polymers and drug molecules were identified. Peak shifting and peak broadening confirmed successful drug encapsulation and polymer–drug interaction.

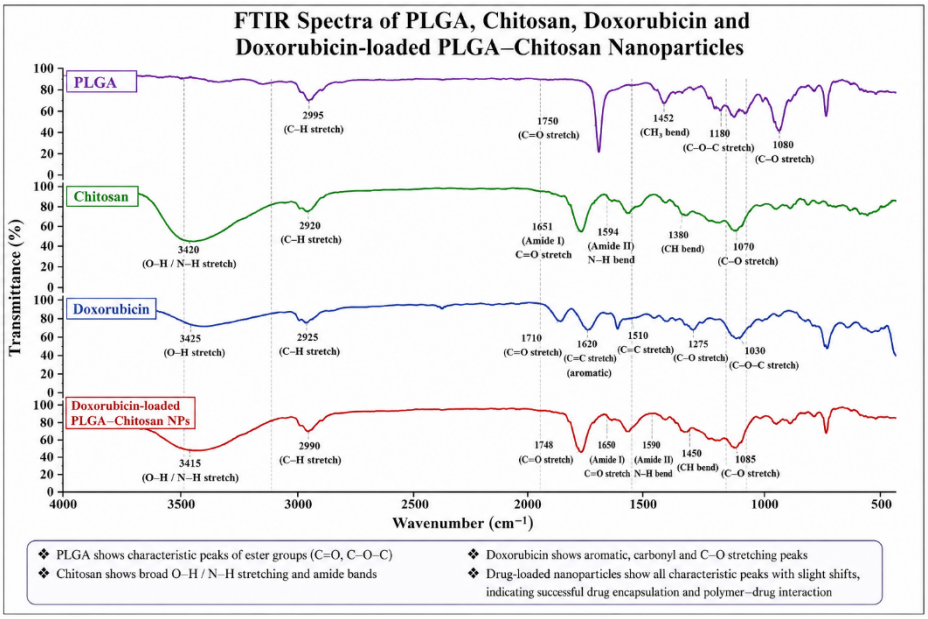

Figure 2 FTIR spectra of PLGA, Chitosan, Doxorubicin, and drug-loaded nanoparticles

2.5.2 Dynamic Light Scattering (DLS) and Zeta Potential

Dynamic Light Scattering analysis was carried out to determine particle size distribution and polydispersity index (PDI) of the nanoparticles. The optimized nanoparticles showed particle size within the nanoscale range suitable for targeted drug delivery applications. Zeta potential analysis was performed to evaluate surface charge and colloidal stability of nanoparticles.

Table 3. Particle size and zeta potential analysis

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	165 ± 4	0.28	-18.4
F2	142 ± 3	0.22	-24.1
F3	178 ± 5	0.31	-20.7

2.5.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy was used to evaluate surface morphology and structural characteristics of the nanoparticles. SEM images demonstrated spherical nanoparticles with smooth surface morphology and uniform distribution. Minimal aggregation confirmed stability of the optimized formulation.

2.5.4 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy analysis was performed to observe internal morphology and nanoscale structure of nanoparticles. TEM images confirmed formation of uniformly dispersed spherical nanoparticles with well-defined boundaries and nanoscale dimensions.

2.6 Drug Loading and Entrapment Efficiency

Drug loading and entrapment efficiency were determined using UV–Visible spectroscopy. After centrifugation, the amount of free Doxorubicin present in the supernatant was measured spectrophotometrically at the characteristic absorption wavelength of the drug.

Drug loading and entrapment efficiency were calculated using standard equations:

Drug Loading (%) = (Amount of drug in nanoparticles / Total nanoparticle weight) × 100

Entrapment Efficiency (%) = (Encapsulated drug / Total drug added) × 100

Table 4. Drug loading and entrapment efficiency

Formulation	Drug Loading (%)	Entrapment Efficiency (%)
F1	63.2	78.5
F2	71.8	86.3
F3	68.4	81.7

2.7 In-vitro Drug Release Study

The in-vitro drug release study was performed using phosphate-buffered saline (PBS, pH 7.4) at 37°C under continuous stirring conditions. Drug-loaded nanoparticles were suspended in release medium and aliquots were withdrawn at predetermined time intervals. The withdrawn samples were analysed using UV–Visible spectroscopy to determine cumulative drug release.

The release profile demonstrated controlled and sustained release behaviour of Doxorubicin from the PLGA–Chitosan nanoparticle system.

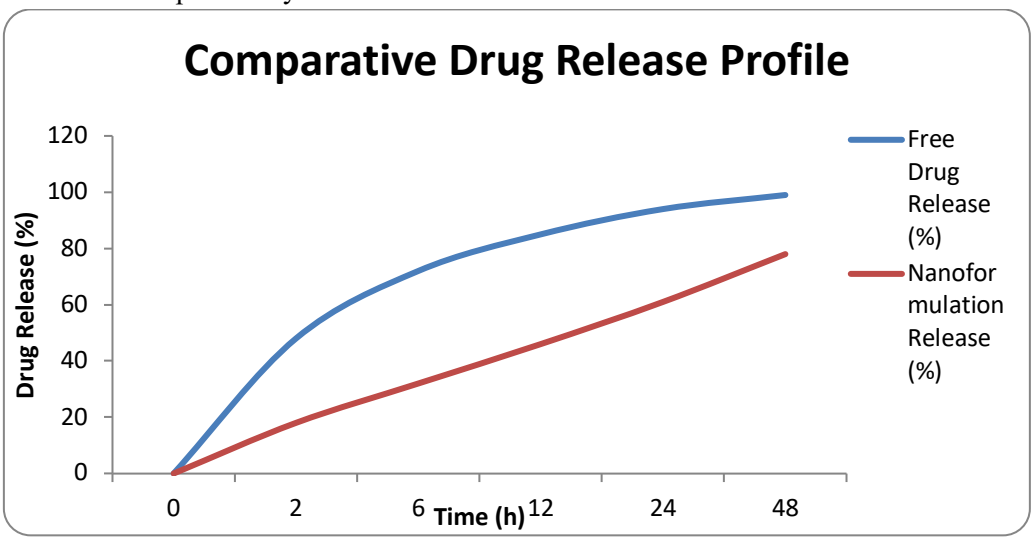

Figure 3 In-vitro cumulative drug release profile

2.8 Drug Release Kinetic Models

Drug release data obtained from in-vitro studies were fitted into different kinetic models including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models to understand the mechanism of drug release.

The Higuchi model indicated diffusion-controlled release behaviour, while the Korsmeyer–Peppas model was used to determine the release exponent and mechanism of diffusion.

2.9 In-vitro Cytotoxicity Assay

The cytotoxicity of the developed nanoformulation was evaluated using the MTT assay on cancer cell lines. Cells were cultured under controlled laboratory conditions and treated with free Doxorubicin and Doxorubicin-loaded nanoparticles at different concentrations.

After incubation, MTT reagent was added and absorbance was measured using a microplate reader. Reduction in cell viability indicated anti-cancer activity of the formulation. The nanoformulation exhibited enhanced cytotoxicity and lower IC_{50} values compared to free Doxorubicin.

Table 5. IC_{50} comparison between free drug and nanoformulation

Sample	IC_{50} (µg/mL)
Free Doxorubicin	8.6
Nanoformulation	4.2

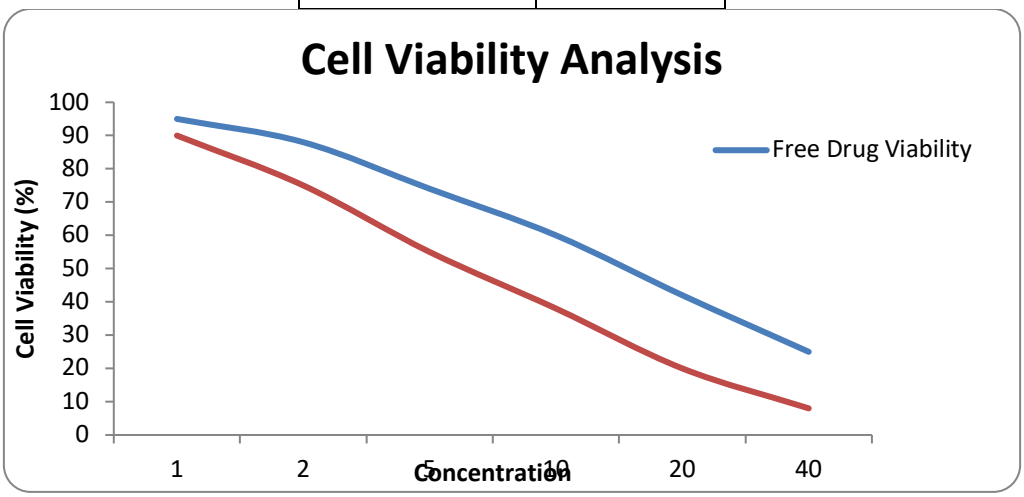

Figure 4 Cytotoxicity graph showing cell viability percentage

2.10 Statistical Analysis

All experimental studies were performed in triplicate and data were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way analysis of variance (ANOVA). Differences between formulations were considered statistically significant at $p < 0.05$. Statistical evaluation was performed to validate reproducibility, reliability, and significance of the obtained results.

3. RESULTS AND DISCUSSION

3.1 Synthesis and Optimization of Nanoparticles

PLGA–Chitosan polymeric nanoparticles were successfully synthesized using the nano-precipitation technique. The method produced stable nanoparticles with uniform particle distribution and satisfactory encapsulation efficiency. Optimization of formulation parameters including polymer concentration, surfactant concentration, stirring speed, and drug-to-polymer ratio significantly influenced nanoparticle formation and physicochemical properties.

Among all formulations, formulation F2 exhibited the most desirable characteristics with minimum particle size, narrow polydispersity index, and maximum drug entrapment efficiency. Higher polymer concentration increased nanoparticle size due to increased solution viscosity, whereas insufficient polymer concentration resulted in unstable particles with poor encapsulation efficiency. Similarly, higher stirring speed improved solvent diffusion and produced smaller nanoparticles with better uniformity.

Table 6. Optimization results of nanoparticle formulations

Formulation	Particle Size (nm)	PDI	Entrapment Efficiency (%)
F1	165 ± 4	0.28	78.5
F2	142 ± 3	0.22	86.3
F3	178 ± 5	0.31	81.7

The optimized nanoparticle formulation showed excellent colloidal stability and nanoscale particle size suitable for targeted anti-cancer drug delivery.

3.2 FTIR Analysis of Drug–Polymer Interaction

FTIR analysis was performed to confirm successful encapsulation of Doxorubicin within the PLGA–Chitosan nanoparticle matrix and to evaluate drug–polymer interaction. The FTIR spectrum of pure PLGA exhibited characteristic absorption peaks corresponding to C=O stretching around 1750 cm^{-1} and C–O stretching near 1080 cm^{-1}. Chitosan showed characteristic peaks related to amino and hydroxyl functional groups around 3400 cm^{-1} and 1650 cm^{-1}.

Doxorubicin exhibited characteristic peaks corresponding to aromatic and hydroxyl groups. In the FTIR spectrum of drug-loaded nanoparticles, slight peak shifting and peak broadening were observed, indicating successful interaction between PLGA, Chitosan, and Doxorubicin molecules. The absence of additional peaks suggested compatibility between polymers and drug molecules without significant chemical degradation.

The observed spectral changes confirmed successful drug encapsulation within the polymeric matrix and stability of the developed nanoformulation.

Figure 10: FTIR spectra of PLGA, Chitosan, Doxorubicin, and Doxorubicin-loaded PLGA–Chitosan nanoparticles

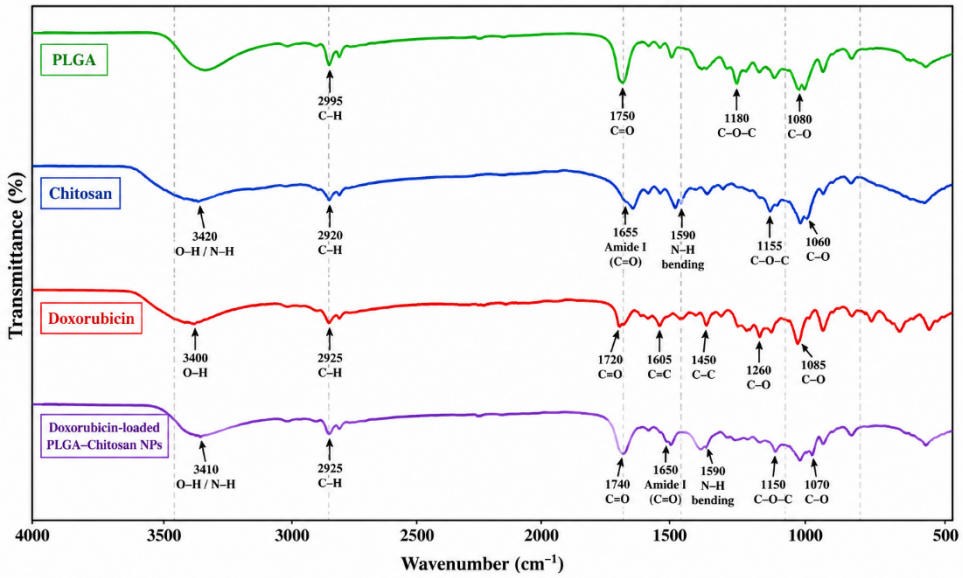

Figure 5 FTIR spectra of PLGA, Chitosan, Doxorubicin, and drug-loaded nanoparticles

3.3 Particle Size, PDI and Zeta Potential Analysis

Dynamic Light Scattering analysis revealed that the synthesized nanoparticles possessed nanoscale particle size distribution suitable for targeted drug delivery applications. The optimized formulation (F2) showed an average particle size of 142 ± 3 nm with a low polydispersity index of 0.22, indicating narrow and uniform particle distribution.

The nanoscale particle size is highly advantageous for cancer therapy because nanoparticles below 200 nm can effectively accumulate within tumour tissues through the enhanced permeability and retention

(EPR) effect. Low PDI values confirmed homogeneity and reduced aggregation tendency of the nanoparticle system.

Zeta potential analysis demonstrated that the nanoparticles possessed stable surface charge values ranging from -18 mV to -24 mV. The optimized formulation showed zeta potential of -24.1 mV, indicating good colloidal stability due to electrostatic repulsion between particles.

Table 7. Particle size and zeta potential analysis

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	165 ± 4	0.28	-18.4
F2	142 ± 3	0.22	-24.1
F3	178 ± 5	0.31	-20.7

The results confirmed that the optimized formulation possessed ideal physicochemical properties required for stable and efficient anti-cancer drug delivery.

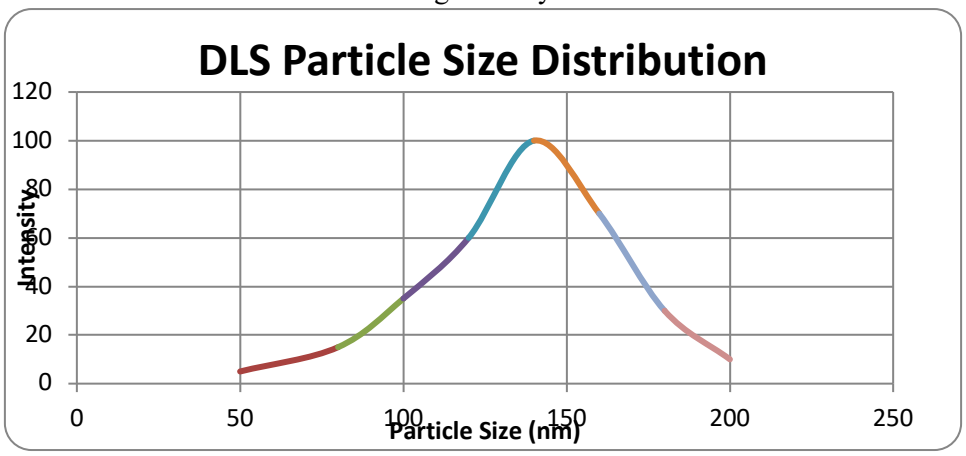

Figure 6 DLS particle size distribution graph

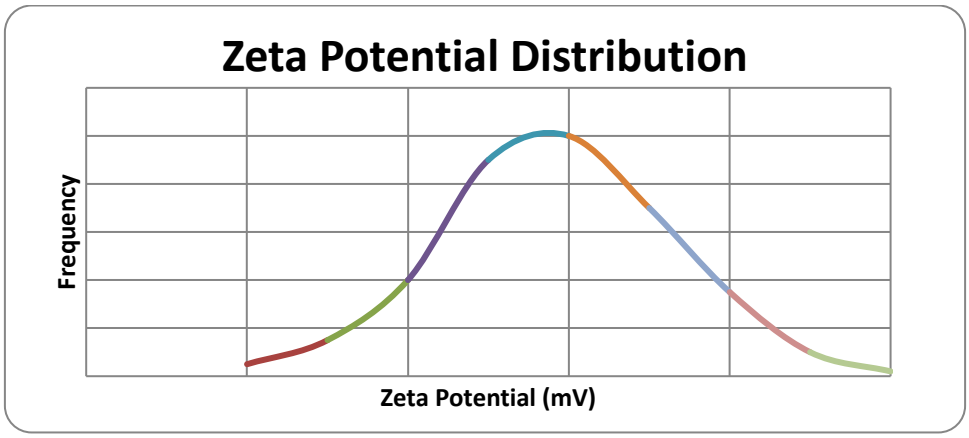

Figure 7 Zeta potential graph

3.4 Surface Morphology Analysis using SEM and TEM

Surface morphology of the synthesized nanoparticles was evaluated using SEM and TEM analysis. SEM images demonstrated spherical nanoparticles with smooth surface morphology and minimal aggregation. Uniform particle distribution confirmed successful stabilization of nanoparticles using PVA surfactant.

TEM analysis further confirmed nanoscale particle size and internal structural integrity of the nanoparticles. The nanoparticles appeared spherical with well-defined boundaries and homogeneous morphology. The observed particle size from TEM analysis correlated well with DLS results.

The spherical morphology and smooth surface characteristics are advantageous for enhanced cellular uptake and controlled drug release behaviour. Minimal aggregation observed in SEM and TEM analysis also indicated excellent colloidal stability of the optimized formulation.

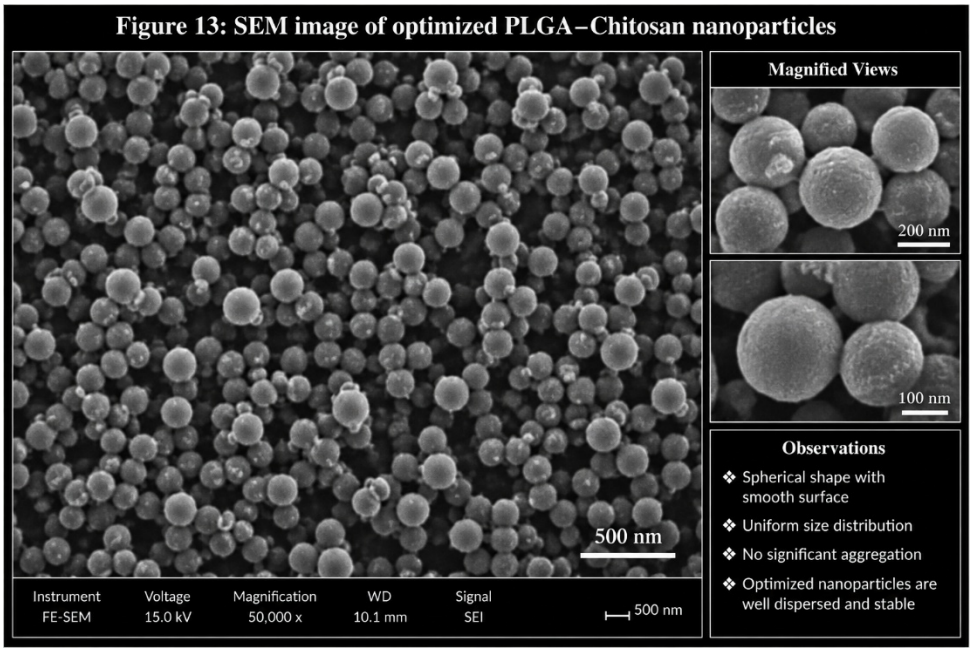

Figure 8 SEM image of optimized nanoparticles

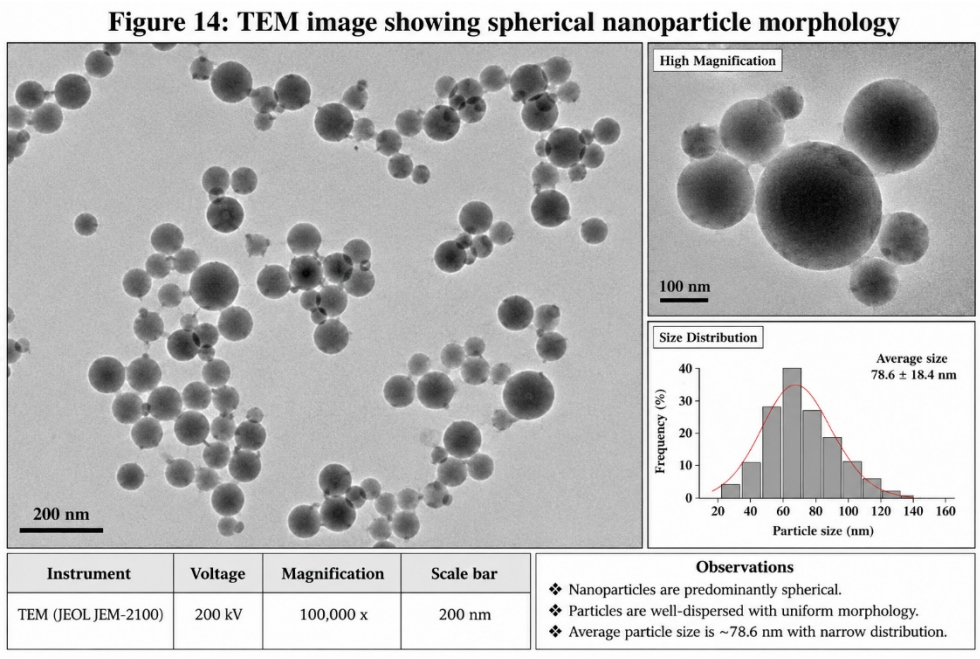

Figure 9 TEM image showing spherical nanoparticle morphology

3.5 Drug Loading and Entrapment Efficiency Results

Drug loading and entrapment efficiency studies demonstrated efficient incorporation of Doxorubicin within the PLGA–Chitosan polymeric matrix. The optimized formulation showed drug loading efficiency of 71.8% and entrapment efficiency of 86.3%, indicating strong encapsulation capability of the polymeric nanoparticles.

Higher entrapment efficiency may be attributed to strong interaction between Doxorubicin and polymeric components during nanoparticle formation. Chitosan coating also contributed to improved drug retention and stabilization within the nanoparticle matrix.

Table 8. Drug loading and entrapment efficiency results

Formulation	Drug Loading (%)	Entrapment Efficiency (%)
F1	63.2	78.5
F2	71.8	86.3
F3	68.4	81.7

The high entrapment efficiency observed in the optimized formulation is highly beneficial for sustained and targeted anti-cancer drug delivery applications.

3.6 In-vitro Controlled Drug Release Behaviour

The in-vitro drug release study demonstrated controlled and sustained release behaviour of Doxorubicin from the PLGA–Chitosan nanoparticle system. Initial burst release was observed during the first few hours due to release of surface-adsorbed drug molecules, followed by gradual and sustained release over 48 hours.

The optimized formulation exhibited approximately 78% cumulative drug release within 48 hours, whereas conventional free Doxorubicin showed rapid release within a shorter duration. Sustained release behaviour of nanoparticles can be attributed to diffusion of Doxorubicin through the polymeric matrix and gradual degradation of PLGA.

Table 9. In-vitro cumulative drug release profile

Time (hours)	Free Drug Release (%)	Nanoformulation Release (%)
2	48	18
6	72	32
12	85	46
24	94	61
48	99	78

Controlled release behaviour is highly advantageous in cancer therapy because it maintains therapeutic drug concentration for prolonged duration while minimizing systemic toxicity and dosing frequency.

3.7 Release Kinetics and Mechanism Analysis

Drug release data obtained from in-vitro studies were fitted into different kinetic models including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models to understand the release mechanism of Doxorubicin from nanoparticles.

Among all models, the Higuchi model showed the highest correlation coefficient, indicating diffusion-controlled release behaviour from the polymeric matrix. Korsmeyer–Peppas analysis suggested anomalous non-Fickian diffusion, indicating that both diffusion and polymer degradation contributed to sustained drug release.

Table 10. Drug release kinetic model analysis

Kinetic Model	Correlation Coefficient (R^2)
Zero-order	0.921
First-order	0.884
Higuchi	0.978
Korsmeyer–Peppas	0.963

The kinetic analysis confirmed that PLGA–Chitosan nanoparticles provided controlled and prolonged release of Doxorubicin suitable for targeted anti-cancer therapy.

3.8 Cytotoxicity and Anti-Cancer Activity Evaluation

The anti-cancer activity of the developed nanoformulation was evaluated using MTT cytotoxicity assay on cancer cell lines. The Doxorubicin-loaded nanoparticles demonstrated significantly enhanced cytotoxicity compared to free Doxorubicin.

The optimized nanoformulation exhibited lower IC_{50} value of 4.2 µg/mL compared to 8.6 µg/mL for free Doxorubicin, indicating improved therapeutic efficiency and enhanced cellular uptake of the nanoparticle system.

Table 11. Cytotoxicity analysis and IC_{50} values

Sample	IC_{50} (µg/mL)
Free Doxorubicin	8.6
Nanoformulation	4.2

The enhanced anti-cancer activity may be attributed to improved cellular internalization, sustained drug release, and efficient tumour targeting properties of the nanoparticle system.

3.9 Comparative Analysis with Conventional Doxorubicin

Comparative evaluation demonstrated that the nanoparticle-mediated drug delivery system significantly improved therapeutic performance compared to conventional Doxorubicin therapy. Free Doxorubicin showed rapid release, lower stability, and reduced cellular uptake, whereas the PLGA–Chitosan nanoformulation exhibited controlled release, improved stability, and enhanced cytotoxic activity.

The nanoformulation also reduced rapid systemic exposure and demonstrated potential for minimizing adverse side effects associated with conventional chemotherapy. Improved drug retention within tumour cells further enhanced therapeutic efficiency of the developed system.

Table 12. Comparative analysis between conventional and nanoparticle-mediated drug delivery

Parameter	Free Doxorubicin	Nanoformulation
Drug Release	Rapid	Controlled
Stability	Moderate	High
IC_{50}	8.6 µg/mL	4.2 µg/mL
Targeting Efficiency	Low	High
Systemic Toxicity	High	Reduced

3.10 Discussion on Targeted Drug Delivery Efficiency

The developed PLGA–Chitosan nanoparticle system demonstrated excellent potential for targeted anti-cancer drug delivery. The nanoscale particle size, stable zeta potential, high drug loading efficiency, and controlled release behaviour collectively contributed to improved therapeutic performance.

The optimized nanoparticles possessed ideal physicochemical properties required for efficient tumour accumulation through the enhanced permeability and retention effect. Chitosan coating enhanced cellular interaction and uptake, while PLGA provided sustained release and structural stability.

The significantly lower IC_{50} value and prolonged release behaviour confirmed improved anti-cancer activity of the nanoformulation compared to conventional Doxorubicin therapy. Overall, the results indicate that PLGA–Chitosan polymeric nanoparticles represent a promising nanotechnology-based platform for targeted and controlled anti-cancer drug delivery applications.

4. CONCLUSION

The present study successfully demonstrated the synthesis, characterization, and evaluation of PLGA–Chitosan polymeric nanoparticles as an efficient drug delivery system for targeted Doxorubicin delivery in cancer therapy. The nano-precipitation method employed for nanoparticle preparation produced

stable and uniformly distributed nanoparticles with desirable physicochemical properties suitable for anti-cancer drug delivery applications.

Optimization studies revealed that formulation parameters such as polymer concentration, surfactant concentration, stirring speed, and drug-to-polymer ratio significantly influenced nanoparticle formation, particle size distribution, and encapsulation efficiency. The optimized formulation exhibited nanoscale particle size with low polydispersity index and stable zeta potential, indicating excellent colloidal stability and suitability for targeted tumour accumulation through the enhanced permeability and retention effect.

Characterization studies using FTIR, DLS, SEM, and TEM confirmed successful encapsulation of Doxorubicin within the PLGA–Chitosan polymeric matrix. FTIR analysis demonstrated effective drug–polymer interaction, while SEM and TEM images confirmed spherical morphology and uniform nanoparticle distribution. DLS analysis further verified nanoscale particle size and stable surface charge properties.

Drug loading and entrapment efficiency studies showed efficient incorporation of Doxorubicin within the nanoparticle system. The developed nanoformulation exhibited controlled and sustained drug release behaviour over an extended period compared to conventional free drug release. Release kinetic analysis confirmed diffusion-controlled release mechanism governed by polymeric matrix diffusion and gradual polymer degradation.

In-vitro cytotoxicity evaluation demonstrated enhanced anti-cancer activity of the Doxorubicin-loaded nanoparticles compared to conventional Doxorubicin. The nanoformulation showed lower IC_{50} values, improved cellular uptake, and enhanced therapeutic efficiency, indicating the potential of PLGA–Chitosan nanoparticles in improving cancer treatment outcomes while reducing systemic toxicity associated with conventional chemotherapy.

Comparative analysis confirmed that nanoparticle-mediated drug delivery provides several advantages including controlled drug release, improved stability, enhanced targeting efficiency, and reduced adverse effects. The combination of PLGA and Chitosan successfully integrated the benefits of synthetic and natural polymers, resulting in a biocompatible and efficient nano-carrier system for anti-cancer drug delivery.

Overall, the findings of this study suggest that PLGA–Chitosan polymeric nanoparticles represent a promising nanotechnology-based platform for targeted and controlled delivery of anti-cancer drugs. The developed formulation has significant potential for improving therapeutic efficacy and minimizing systemic toxicity in cancer therapy. Further in-vivo investigations and clinical studies are recommended to validate the practical applicability and long-term therapeutic performance of the developed nanoparticle system in advanced cancer treatment.

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