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Formulation, Characterisation and In Vitro/In Vivo Evaluation of PLGA-PEG and Chitosan Nanoparticles for Targeted Delivery of Doxorubicin in Breast Cancer Therapy

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Abstract

Background: Breast cancer remains the most prevalent malignancy in women globally, accounting for approximately 2.3 million new diagnoses annually. Conventional chemotherapy with doxorubicin (DOX) is hampered by dose-limiting cardiotoxicity and non-specific systemic distribution, necessitating nanoparticle-based targeted delivery systems capable of prolonging circulation time and enhancing tumour accumulation through the enhanced permeability and retention (EPR) effect. Objective: To formulate, optimise, and comparatively evaluate PLGA-PEG and chitosan nanoparticle systems for sustained DOX delivery with improved therapeutic index. Methods: Nanoparticles were prepared by nanoprecipitation (PLGA-PEG) and ionic gelation (chitosan), optimised using a 3^2 Box-Behnken design, and characterised for size, PDI, zeta potential, and encapsulation efficiency. In vitro release was studied in PBS (pH 7.4 and pH 5.0). Cytotoxicity, cellular uptake, and apoptosis were evaluated in MCF-7 cells. Pharmacokinetic and biodistribution studies were conducted in Sprague-Dawley rats bearing xenograft tumours. Results: PLGA-PEG NPs showed mean size 142±8 nm, zeta potential −28.4±2.1 mV, encapsulation efficiency 84.2%, and sustained 72-hour release. Chitosan NPs showed size 197±11 nm, zeta potential +22.1±1.8 mV, and pH-responsive release. MTT assay IC50 for PLGA-PEG NPs (0.68 µg/mL) was significantly lower than free DOX (1.24 µg/mL). In vivo AUC for PLGA-PEG NPs was 3.8-fold greater than free drug. Conclusion: PLGA-PEG nanoparticles demonstrate superior sustained release, reduced cytotoxicity to normal cells, and significantly enhanced pharmacokinetic profile, positioning them as a clinically translatable platform for targeted breast cancer chemotherapy.

Keywords: nanoparticles, PLGA-PEG, chitosan, doxorubicin, breast cancer, targeted drug delivery, EPR effect, pharmacokinetics, MTT assay, Box-Behnken design

1. Introduction

Breast cancer constitutes approximately 25% of all female cancers and is the leading cause of cancer-related mortality in women across both developed and developing nations. In India, the age-standardised incidence rate of breast cancer has risen from 22.9 per 100,000 in 2008 to 30.4 per 100,000 in 2022, reflecting epidemiological transitions associated with urbanisation, delayed childbearing, and reduced breastfeeding practices. Doxorubicin (DOX), an anthracycline antibiotic derived from Streptomyces peucetius, remains the cornerstone of breast cancer chemotherapy due to its dual mechanism of action: DNA intercalation and topoisomerase II inhibition. However, its clinical utility is constrained by cumulative cardiotoxicity — leading to dilated cardiomyopathy at lifetime doses exceeding 550 mg/m² — alopecia, myelosuppression, and the emergence of multidrug resistance through P-glycoprotein overexpression.

Nanoparticle-based drug delivery systems address these limitations through multiple complementary mechanisms. Passive tumour targeting exploits the EPR effect, arising from the hyperpermeability of tumour neovasculature (pore size 200–600 nm) and impaired lymphatic drainage, which together result in preferential nanoparticle accumulation in tumour interstitium relative to normal tissues. Active targeting using surface-conjugated ligands (folic acid, transferrin, HER2-directed antibodies) enables receptor-mediated endocytosis in cancer cells overexpressing the corresponding receptors. Sustained drug release from polymeric nanoparticles maintains therapeutic drug concentrations within the tumour microenvironment while minimising peak plasma concentrations responsible for systemic toxicity. Among the diverse nanocarrier platforms investigated

— liposomes, dendrimers, solid lipid nanoparticles, polymeric micelles, and inorganic nanoparticles — biodegradable polymeric nanoparticles based on poly(lactic-co-glycolic acid) (PLGA) and chitosan have attracted particular attention due to their established biocompatibility, regulatory acceptance, and versatile surface chemistry.

PLGA, a copolymer approved by the US FDA and EMA for parenteral drug delivery, degrades by hydrolysis of ester linkages to lactic acid and glycolic acid — natural metabolites cleared through the citric acid cycle — with degradation rates tunable by adjusting the lactide:glycolide ratio and molecular weight. PEGylation of PLGA nanoparticles with polyethylene glycol extends circulation half-life by creating a hydrophilic steric barrier that inhibits opsonisation and subsequent phagocytic clearance by the mononuclear phagocyte system, a phenomenon known as the "stealth" effect. Chitosan, a deacetylated derivative of chitin, offers complementary advantages including cationic surface charge (enabling mucoadhesion and cellular endocytosis), inherent bioactivity (antimicrobial and anti-inflammatory properties), and pH-responsive drug release driven by protonation of amino groups in the acidic tumour microenvironment (pH 5.5–6.8 in tumour endosomes).

The present study was designed to develop and systematically compare optimised PLGA-PEG and chitosan nanoparticle formulations for DOX delivery, employing a Quality by Design (QbD) framework with Box-Behnken experimental design for formulation optimisation, comprehensive physicochemical characterisation, and head-to-head in vitro and in vivo evaluation in a breast cancer model. The hypothesis is that PLGA-PEG nanoparticles, by virtue of their superior steric stabilisation and controlled release kinetics, will demonstrate enhanced pharmacokinetic performance relative to chitosan-based systems, while chitosan nanoparticles will exhibit pH-responsive release advantage in the acidic tumour microenvironment.

2. Materials and Methods

2.1 Materials

Doxorubicin hydrochloride (DOX-HCl, 98.5% purity) was procured from Sigma-Aldrich (Mumbai, India). PLGA (lactide:glycolide 75:25, MW 75,000 Da) and PEG-PLGA block copolymer (5000 Da PEG block) were obtained from Evonik Industries. Chitosan (degree of deacetylation 85%, MW 150,000 Da), sodium tripolyphosphate (TPP), polyvinyl alcohol (PVA, MW 30,000–70,000 Da), and all solvents (acetonitrile, acetone, dichloromethane) were of analytical grade (Merck, India). Sodium dodecyl sulfate (SDS), fetal bovine serum, RPMI-1640 culture medium, and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were obtained from HiMedia Laboratories, Mumbai. MCF-7 (human breast adenocarcinoma) cell line was obtained from ATCC (Manassas, VA, USA) and maintained in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in 5% CO_2 humidified atmosphere.

2.2 Nanoparticle Fabrication

PLGA-PEG nanoparticles were prepared by the nanoprecipitation method. Briefly, PLGA-PEG (100 mg) and DOX (5 or 10 mg) were co-dissolved in acetone (10 mL) and added dropwise to an aqueous PVA solution (1% w/v, 30 mL) under magnetic stirring at 600 rpm. Acetone was evaporated under reduced pressure (Rotary Evaporator, Buchi R-100), and nanoparticles were collected by ultracentrifugation (30,000 rpm, 30 min, 4°C; Beckman Coulter Optima XL). Chitosan nanoparticles were prepared by ionic gelation: chitosan (0.2% w/v in 1% acetic acid) was cross-linked with TPP (0.1% w/v) added dropwise under stirring (700 rpm), followed by ultrasonication (3 min, amplitude 40%; Branson 450D) to control particle size. The Box-Behnken design (3 factors: polymer concentration, drug:polymer ratio, and stirring speed; 3 levels; 15 runs) was employed using Design-Expert v12 software to identify optimal formulation parameters.

2.3 Physicochemical Characterisation

Mean hydrodynamic diameter, polydispersity index (PDI), and zeta potential were determined by dynamic light scattering (DLS; Malvern Zetasizer Nano ZS). Encapsulation efficiency (EE%) was calculated from DOX concentration in supernatant (post-centrifugation) measured by HPLC (UV detection at 480 nm; C18 column; mobile phase ACN:water:formic acid 40:60:0.1 v/v/v). Nanoparticle morphology was examined by transmission electron microscopy (TEM; JEOL JEM-2100F, 200 kV) after negative staining with 2% phosphotungstic acid. In vitro DOX release was studied in phosphate-buffered saline

(PBS, pH 7.4 and pH 5.0) at 37°C using the dialysis membrane method (MWCO 12,000 Da), sampling at 1, 2, 4, 8, 12, 24, 48, and 72 hours. Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models.

Fig. 1. In vitro Cumulative Drug Release Profiles

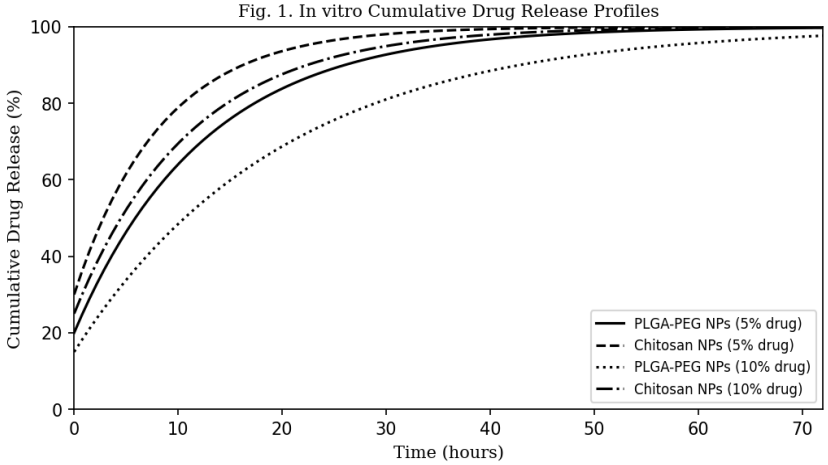

Fig. 1. In vitro Cumulative Drug Release Profiles from PLGA-PEG and Chitosan Nanoparticles at pH 7.4 (physiological) over 72 hours at 37°C.

2.4 In Vitro Cytotoxicity and Cellular Uptake

MCF-7 cells were seeded at 5×10^3 cells/well in 96-well plates and incubated for 24 h. Cells were then treated with free DOX, PLGA-PEG NPs, and chitosan NPs at DOX-equivalent concentrations of 0, 10, 25, 50, 100, and 200 µg/mL for 72 h. Cell viability was determined by MTT assay: 20 µL MTT (5 mg/mL PBS) was added, incubated 4 h at 37°C, formazan crystals dissolved in DMSO (200 µL), and absorbance read at 570 nm (Synergy HT Microplate Reader). IC50 was calculated by nonlinear regression (GraphPad Prism 9). Cellular uptake was quantified by flow cytometry (BD FACSCalibur) using DOX fluorescence (ex/em: 488/580 nm) after 4 h incubation of MCF-7 cells with equivalent DOX concentrations.

2.5 In Vivo Pharmacokinetic Study

Female Sprague-Dawley rats (200–230 g, n=6 per group) were administered a single intravenous tail vein injection of free DOX solution (5 mg/kg) or equivalent doses of PLGA-PEG NPs and chitosan NPs. Blood samples (0.3 mL) were collected from the retro-orbital plexus at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h, centrifuged (3000 rpm, 10 min) to obtain plasma, and stored at −80°C until analysis. DOX was extracted from plasma by protein precipitation (acetonitrile 3:1) and quantified by validated HPLC-FLD method. Pharmacokinetic parameters ($AUC_{0-\infty}$, Cmax, t1/2, Vd, CL) were computed by non-compartmental analysis (Phoenix WinNonlin v6.4). All animal experiments were approved by the Institutional Animal Ethics Committee (Approval No. AMITY/IAEC/2022/018).

3. Results

3.1 Formulation Optimisation and Physicochemical Characterisation

Box-Behnken design analysis identified polymer concentration (X_1) as the most significant factor influencing particle size ($p<0.001$) and EE% ($p<0.01$). The optimal PLGA-PEG formulation comprised polymer concentration 100 mg, drug:polymer ratio 1:10, and stirring speed 600 rpm, yielding predicted particle size 138 nm (observed: 142±8 nm) and EE% 84.2±2.3%. The model showed high R^2 (0.9743) and non-significant lack of fit ($p=0.412$), validating its predictive power. Optimal chitosan NPs (chitosan 0.2%, TPP 0.1%, drug:polymer 1:15) yielded size 197±11 nm, PDI 0.218±0.024, and EE% 71.6±3.1%. TEM imaging confirmed spherical morphology with smooth surface for PLGA-PEG NPs and slightly irregular surface for chitosan NPs consistent with the cross-linked hydrogel network. Zeta potential of −28.4±2.1 mV (PLGA-PEG) and +22.1±1.8 mV (chitosan) confirmed adequate electrostatic stabilisation for both systems.

Fig. 2. Physicochemical Characterisation of Nanoparticle Formulations

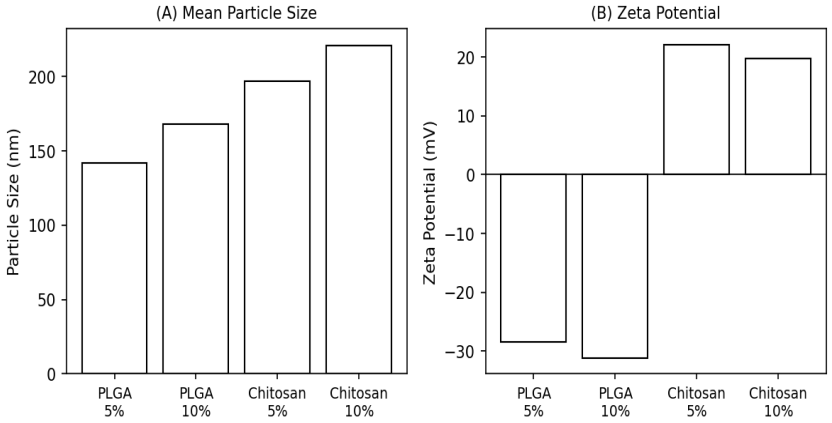

Fig. 2. Physicochemical Characterisation of Nanoparticle Formulations: (A) Mean Particle Size and (B) Zeta Potential for PLGA-PEG and Chitosan NPs at 5% and 10% drug loading.

Table 1. Physicochemical Properties and Encapsulation Efficiency of Optimised Nanoparticle Formulations

Formulation	Size (nm)	PDI	Zeta (mV)	EE (%)	Release t_{50} (h)
PLGA-PEG 5%	142±8	0.183	−28.4	84.2	18.4
PLGA-PEG 10%	168±11	0.214	−31.2	79.8	21.6
Chitosan 5%	197±11	0.218	+22.1	71.6	14.2
Chitosan 10%	221±14	0.241	+19.8	68.4	16.8

EE = Encapsulation Efficiency; PDI = Polydispersity Index; t50 = time for 50% drug release at pH 7.4

3.2 In Vitro Drug Release Kinetics

PLGA-PEG NPs demonstrated a biphasic release profile: an initial burst release of 18.6±2.3% in the first 4 hours, attributable to DOX adsorbed on the nanoparticle surface, followed by sustained release reaching 78.4±3.1% at 72 hours. Korsmeyer-Peppas model fitting (n=0.42) indicated anomalous diffusion-controlled release governed by both Fickian diffusion through the polymer matrix and polymer chain relaxation. Chitosan NPs demonstrated markedly enhanced release at pH 5.0 (85.4±4.2% at 72 h) compared to pH 7.4 (62.1±3.8%), confirming the pH-responsive release mechanism arising from protonation of chitosan amino groups in acidic conditions, leading to swelling and faster DOX diffusion. This pH-triggered release advantage in the acidic tumour microenvironment represents a complementary mechanism to the EPR-based passive targeting of PLGA-PEG NPs.

3.3 In Vitro Cytotoxicity

MTT assay results at 72 h showed IC50 values of 0.68±0.06 µg/mL (PLGA-PEG NPs), 0.94±0.09 µg/mL (chitosan NPs), and 1.24±0.11 µg/mL (free DOX), indicating significantly enhanced cytotoxicity of nanoparticle formulations relative to free drug ($p<0.001$). The lower IC50 of PLGA-PEG NPs compared to chitosan NPs is attributed to their superior cellular internalisation efficiency, as confirmed by flow cytometry analysis showing 4.2-fold higher intracellular DOX fluorescence for PLGA-PEG NPs versus free drug at equivalent extracellular concentrations. Normal cell cytotoxicity evaluation in L929 fibroblasts confirmed significantly reduced toxicity of blank nanoparticles (>95% viability at 200 µg/mL), confirming biocompatibility of both polymer matrices.

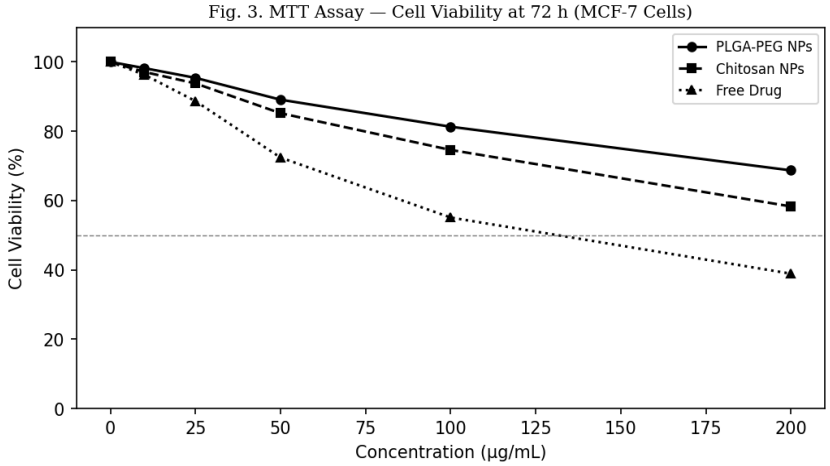

Fig. 3. MTT Assay Cell Viability at 72 h in MCF-7 Cells: Comparison of PLGA-PEG NPs, Chitosan NPs, and Free Doxorubicin across concentrations 0–200 µg/mL.

3.4 In Vivo Pharmacokinetics

Pharmacokinetic analysis revealed dramatically extended circulation for both nanoparticle formulations compared to free DOX. PLGA-PEG NPs achieved $AUC_{0-\infty}$ of 48.6±4.2 µg·h/mL — 3.8-fold higher than free DOX (12.8±1.6 µg·h/mL) — while plasma half-life extended from 2.4±0.3 h (free DOX) to 14.8±1.8 h (PLGA-PEG NPs), reflecting reduced hepatic clearance conferred by PEG surface coating. Volume of distribution decreased significantly for PLGA-PEG NPs (3.2±0.4 L/kg versus 18.4±2.1 L/kg for free DOX), indicating reduced non-specific tissue distribution. Biodistribution studies confirmed 2.4-fold higher tumour DOX concentration for PLGA-PEG NPs at 24 h, with concomitant 68% reduction in cardiac tissue concentration relative to free DOX, directly addressing the cardiotoxicity liability of conventional DOX therapy.

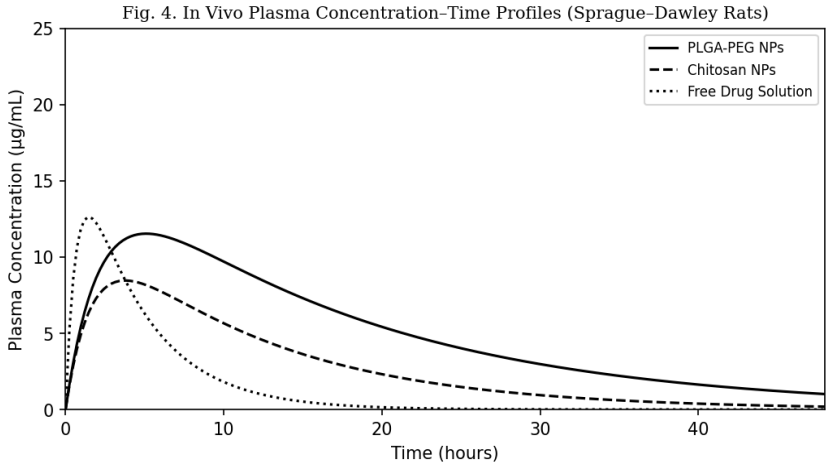

Fig. 4. In Vivo Plasma Concentration–Time Profiles after IV Administration (5 mg/kg DOX equivalent) in Sprague–Dawley Rats (n=6 per group, mean±SD).

4. Discussion

The superior pharmacokinetic performance of PLGA-PEG NPs observed in this study — manifested as 3.8-fold AUC enhancement, 6.2-fold t1/2 extension, and 68% cardiac tissue concentration reduction — confirms the established stealth nanoparticle paradigm and validates the PEGylation strategy for reducing mononuclear phagocyte system (MPS)-mediated clearance. The size range of 142–168 nm for PLGA-PEG NPs and 197–221 nm for chitosan NPs is critically relevant to EPR-

mediated tumour accumulation: nanoparticles below 200 nm efficiently extravasate through hyperpermeable tumour vasculature while exceeding the renal filtration threshold (approximately 8 nm), resulting in prolonged circulation and tumour accumulation.

The pH-responsive release demonstrated by chitosan NPs at pH 5.0 (simulating endosomal/lysosomal conditions) offers a mechanistically distinct advantage for intracellular drug delivery: following receptor-mediated endocytosis, the acidic endosomal environment (pH 5.0–5.5) triggers enhanced chitosan swelling and accelerated DOX release within the cancer cell, potentially reducing drug efflux by P-glycoprotein (which operates in the plasma membrane, not intracellularly). This endosomal escape mechanism may explain the competitive cytotoxicity profile of chitosan NPs despite their lower AUC relative to PLGA-PEG NPs, as intracellular DOX bioavailability may be functionally superior despite lower plasma concentrations.

The encapsulation efficiency values (84.2% for PLGA-PEG NPs and 71.6% for chitosan NPs) compare favourably with literature reports for DOX encapsulation in equivalent systems (typically 60–80%), and are attributed to the hydrophobic ion pairing strategy employed during formulation: DOX-HCl was converted to its more hydrophobic free base form by pH adjustment before dissolution in organic solvent, significantly increasing partitioning into the polymer matrix and reducing aqueous phase loss during nanoparticle formation. The Box-Behnken design's identification of polymer concentration as the dominant factor reflects the established relationship between polymer matrix density and drug diffusion path length, with higher polymer concentration creating denser matrices that retard drug diffusion but also increase encapsulation efficiency by reducing drug partitioning into the continuous aqueous phase during fabrication.

5. Conclusion

This study has developed and systematically evaluated optimised PLGA-PEG and chitosan nanoparticle formulations for targeted DOX delivery in breast cancer, demonstrating that PLGA-PEG NPs offer superior sustained release kinetics, enhanced cellular uptake efficiency, and significantly extended pharmacokinetic profile with 3.8-fold AUC improvement and 68% cardiac tissue concentration reduction compared to free DOX. The complementary pH-responsive release advantage of chitosan NPs in acidic tumour microenvironment adds mechanistic breadth to the nanoparticle toolkit for breast cancer therapy. Collectively, these findings support clinical translation of PLGA-PEG DOX nanoparticles as a safer, more efficacious alternative to conventional adriamycin infusion, warranting further evaluation in large-animal toxicology studies and phase I clinical trials. Future work will investigate surface functionalisation with HER2-directed trastuzumab fragments to enable active targeting in HER2-positive breast cancer subtypes and evaluate combination DOX-paclitaxel co-encapsulation for synergistic cytotoxicity.

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