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Formulation, characterization, and *in vitro* evaluation of chitosan nanoparticles for doxorubicin delivery

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Abstract
Doxorubicin (DOX) remains a cornerstone chemotherapeutic despite dose-limiting cardiotoxicity and poor tumor specificity stemming from rapid clearance and non-selective distribution. This study engineered chitosan nanoparticles (CSNPs) via ionic gelation to leverage the enhanced permeability and retention (EPR) effect for improved targeting. Three formulations (F1-F3) varied chitosan concentrations (0.25-0.75% w/v) with 1% sodium tripolyphosphate (TPP) and 1 mg/mL DOX, yielding particles of 175-193 nm (PDI <0.3), zeta potentials +33 to +38 mV, and entrapment efficiencies (EE) 83.9-89.0%. Dynamic light scattering confirmed monodispersity, while scanning electron microscopy revealed spherical morphology with smooth surfaces. Preformulation studies via Fourier-transform infrared spectroscopy verified drug-excipient compatibility, showing no shifts in characteristic DOX peaks (amide OH/NH at 3400 cm^{-1}, C=O at 1720 cm^{-1}). *In vitro* release at pH 5.5 (tumor mimic) exhibited biphasic kinetics: initial 15-20% burst within 2 hours followed by sustained release reaching 85-90% over 12 hours, best fitting Higuchi diffusion model ($R^2 > 0.98$). Free DOX showed >80% burst release in 4 hours, highlighting CSNP superiority. Optimized F2 (180 nm, 87.2% EE) demonstrates colloidal stability, high drug loading, and pH-responsive release ideal for reducing systemic exposure while enhancing intratumoral accumulation. These biocompatible, biodegradable CSNPs address key DOX limitations, warranting *in vivo* pharmacokinetics and efficacy studies for clinical translation in solid tumor therapy.

Keywords: Chitosan nanoparticles, doxorubicin, ionic gelation, entrapment efficiency, sustained release, EPR effect, cancer nanotherapy

Introduction

Doxorubicin (DOX), an anthracycline antibiotic isolated from Streptomyces peucetius, intercalates DNA, inhibits topoisomerase II, and generates reactive oxygen species (ROS) to induce apoptosis in rapidly proliferating cancer cells. Clinically, DOX treats breast cancer, leukemia, lymphoma, and sarcomas within regimens like CHOP and ABVD, improving survival rates across solid and hematologic malignancies. However, therapeutic utility remains constrained by severe toxicities: cumulative cardiotoxicity exceeds 550 mg/m^2 due to ROS-mediated mitochondrial damage in cardiomyocytes, myelosuppression, alopecia, and extravasation-induced necrosis. Intravenous administration exacerbates these via poor tumor specificity, first-pass metabolism, and short half-life (20-48 hours), necessitating frequent high dosing that amplifies off-target effects.

Nanotechnology addresses these barriers through carriers exploiting the EPR effect—leaky tumor vasculature (200-800 nm pores) permits preferential nanoparticle accumulation while dense lymphatics limit clearance. Polymeric nanoparticles like poly (lactic-co-glycolic acid) (PLGA) and liposomes (Doxil®) extend circulation, reduce peak plasma concentrations, and localize DOX, cutting cardiotoxicity by 50-70% in clinical use. Chitosan, a cationic polysaccharide from chitin deacetylation (MW 100-200 kDa, 85% deacetylation), emerges superior due to biocompatibility, biodegradability via lysozyme, mucoadhesion, and positive charge facilitating electrostatic tumor cell interactions and endosomal escape. Ionic gelation with TPP yields uniform nanoparticles without toxic solvents, enabling high DOX loading (>80% EE) and scalability.

Literature underscores CSNPs' promise: pH-sensitive formulations release 80% DOX under acidic tumor microenvironments (pH 5.5-6.5) versus physiological pH 7.4, enhancing cytotoxicity while sparing healthy tissues. Folic acid conjugation boosts uptake via overexpressed receptors on breast/ovarian cancers. Yet gaps persist—few studies optimize chitosan:TPP ratios for <200 nm sizes balancing stability and release, with limited direct free DOX comparisons. This work hypothesizes CSNPs achieve optimal physicochemical properties (size <200 nm, zeta >+30 mV, EE >85%) and biphasic release superior to free drug, positioning them for EPR-mediated therapy.

Materials and Methods

Doxorubicin hydrochloride (Sigma-Aldrich, USA), low molecular weight chitosan (100-200 kDa, 85% deacetylation, Sigma-Aldrich), sodium tripolyphosphate pentabasic (TPP, HiMedia, India), glacial acetic acid (Merck, India), phosphate-buffered saline (PBS) tablets (HiMedia). All analytical grade; Milli-Q water used.

Preformulation Studies

DOX standard curve prepared (1-10 µg/mL) in PBS; λ_max scanned 200-800 nm (UV-1800, Shimadzu, Japan). Drug-polymer compatibility assessed via FTIR (IRAffinity-1S, Shimadzu): KBr pellets (1% w/w), 4000-400 cm^{-1}, 4 cm^{-1} resolution, 32 scans.

Preparation of DOX-Loaded CSNPs

Ionic gelation method adapted: Chitosan dissolved in 1% v/v acetic acid (magnetic stirring, 2 h, 25°C) to 0.25% (F1), 0.5% (F2), or 0.75% (F3) w/v. DOX (1 mg/mL) added to

chitosan solution (10 mL). TPP (1% w/v, 4 mL) added dropwise under 1000 rpm stirring (30 min, 25°C) for cross-linking. Suspensions centrifuged (REMI cooling centrifuge, 15,000 rpm, 30 min, 4°C), pellets washed thrice (Milli-Q), recentrifuged, lyophilized (24 h, -50°C).

Physicochemical Characterization

Particle size, polydispersity index (PDI), zeta potential measured via dynamic light scattering (DLS; Zetasizer Nano ZS90, Malvern, UK; n=3, 25°C). Morphology via scanning electron microscopy (SEM; JSM-6390LV, JEOL, Japan): gold-sputtered samples, 15 kV. EE calculated post-centrifugation: free DOX in supernatant quantified UV (480 nm); EE% = [(Total DOX – Free DOX)/Total DOX] × 100.

***In Vitro* Drug Release and Kinetics**

Release via dialysis (MWCO 12-14 kDa; 2 mL F2 equivalent, 50 mg NPs) in 50 mL PBS (pH 5.5/7.4, 37±0.5°C, 100 rpm). Aliquots (5 mL) withdrawn (0.5, 1, 2, 4, 6, 8, 12 h), replaced; DOX assayed UV (480 nm). Free DOX control parallel. Cumulative release plotted; kinetics fitted: zero-order ($Q = k_0 t$), first-order ($\ln(100-Q) = -k_1 t$), Higuchi ($Q = k_H \sqrt{t}$), Korsmeyer-Peppas ($\log Q = \log k + n \log t$). Best fit by highest R^2. Data analyzed (mean ± SD, n=3); significance via one-way ANOVA ($p<0.05$).

Results

Preformulation Studies

Doxorubicin (DOX) exhibited a maximum absorbance (λ_{max}) at 480 nm with a molar absorptivity of $\varepsilon = 11{,}500\ M^{-1}\ cm^{-1}$ and a linear calibration curve ($R^2 = 0.999$).

FTIR Analysis:

Characteristic peaks of pure DOX were observed at 3400 cm^{-1} (O–H/N–H stretching), 2930 cm^{-1} (C–H stretching), 1720 cm^{-1} (C=O, amide I), and 1630 cm^{-1} (amide II). These peaks were retained in the physical mixture and DOX-loaded chitosan nanoparticles (CSNPs) without any shifting or formation of new functional groups, indicating no chemical interactions between DOX and the excipients.

Physicochemical Properties

Optimized CSNPs demonstrated concentration-dependent changes across formulations (Table 1). Formulation F2 (0.5% chitosan) produced the most desirable properties:

- Particle size: 180 ± 4 nm
- PDI: 0.28 ± 0.02 (indicating monodispersity)
- Zeta potential: +35 ± 1 mV (stable, electrostatic repulsion)

Formulation F3 (0.75%) produced slightly smaller particles due to increased cross-linking but presented a higher risk of aggregation.

Table 1: Physicochemical Parameters of DOX-CSNPs (n = 3)

Formulation	Chitosan (% w/v)	Size (nm)	PDI	Zeta Potential (mV)	EE (%)
F1	0.25	193 ± 5	0.31 ± 0.03	+33 ± 2	83.9 ± 1.2
F2	0.50	180 ± 4	0.28 ± 0.02	+35 ± 1	87.2 ± 0.8
F3	0.75	175 ± 3	0.25 ± 0.01	+38 ± 2	89.0 ± 1.0

Dynamic light scattering (DLS) showed unimodal distributions, with the main peak around 180 nm, consistent with scanning electron microscopy (SEM), which revealed spherical, smooth-surfaced nanoparticles (170–190 nm; scale: 200 nm).

***In Vitro* Release Studies**

At pH 5.5, F2 exhibited a characteristic biphasic release profile consisting of:

- Initial burst release: 18.5% in 2 h
- Sustained release: up to 90.1% at 12 h

At pH 7.4, total release was lower (72.4% at 12 h), consistent with reduced chitosan swelling at physiological pH.

Free DOX showed rapid release (85.7% at 4 h), confirming loss of control when not encapsulated.

Release kinetics:

- Best fitted by Higuchi model ($R^2 = 0.99$; slope: 9.8 % $h^{-1/2}$)
- Korsmeyer–Peppas exponent n = 0.52, indicating anomalous (Fickian-dominant) diffusion

Table 2: Cumulative Release Profiles (n = 3)

Time (h)	F2 Release (pH 5.5) %	F2 Release (pH 7.4) %	Free DOX (pH 5.5) %
0.5	8.2 ± 0.5	6.1 ± 0.4	45.1 ± 2.1
2	18.5 ± 1.2	14.3 ± 0.9	65.3 ± 3.4
4	35.2 ± 2.0	28.7 ± 1.8	85.7 ± 4.2
8	62.4 ± 3.1	48.2 ± 2.5	92.1 ± 3.8
12	90.1 ± 4.5	72.4 ± 3.9	95.4 ± 2.9

Discussion

The study successfully demonstrated that DOX-loaded CSNPs exhibit highly favorable physicochemical and release characteristics aligned with current nanomedicine requirements. The optimized formulation (F2) achieved a particle size below 200 nm, which is the ideal range for enhanced permeability and retention (EPR) effect–mediated tumor targeting. The narrow PDI (0.28) signifies uniformity essential for reproducibility and large-scale translation. Furthermore, the strong positive zeta potential (+35 mV) ensures robust electrostatic repulsion, minimizing aggregation and prolonging blood circulation time.

The encapsulation efficiency (EE) of 87% markedly surpasses that of traditional polymeric nanoparticles, such as PLGA (60–75%). This enhancement arises from strong electrostatic interactions between cationic chitosan, positively charged DOX, and the multivalent cross-linker TPP^{3-}, forming a stable polyelectrolyte complex. The 0.5% chitosan concentration in F2 provided an optimal balance between viscosity and cross-linking density. In comparison,

higher concentrations (e.g., F3) slightly improved entrapment but risked compromising dispersibility and injectability.

The SEM results corroborated DLS findings by confirming spherical morphology, which supports predictable hydrodynamic behavior and contributes to consistent pharmacokinetics. Chitosan's biodegradability and low immunogenicity further enhance its suitability for clinical translation.

The biphasic release pattern—initial burst followed by sustained release—is highly advantageous for chemotherapy. The burst phase rapidly establishes therapeutic concentrations, while the sustained phase maintains prolonged exposure, reducing dosing frequency and mitigating cardiotoxicity associated with peak DOX levels. The significantly faster release at pH 5.5 compared to pH 7.4 underscores the system's tumor microenvironment responsiveness, leveraging acidic tumor physiology for targeted delivery. Free DOX exhibited uncontrolled, rapid release, highlighting the superiority of nanoparticle encapsulation.

Mathematical modeling confirmed Higuchi kinetics (R^2 = 0.99), characteristic of diffusion-controlled release. The Korsmeyer–Peppas exponent (n = 0.52) indicates quasi-Fickian diffusion, influenced by polymer swelling and erosion. Compared to liposomal DOX formulations like Doxil (100 nm size, ~80% release at 24 h), CSNPs offer additional advantages such as mucoadhesion, lower production cost, and customizable surface chemistry.

Literature evidence reinforces these findings: similar CSNPs have demonstrated enhanced cellular uptake (2-fold increase in MCF-7 cells) and significant tumor suppression in animal models, with reduced cardiotoxic biomarkers (e.g., Troponin I). FTIR stability profiles further confirm the absence of drug–excipient interactions, supporting long-term storage potential.

Despite these strengths, limitations include the lack of *in vivo* biodistribution and cytotoxicity data. Future studies should incorporate PEGylation for improved stealth properties, ligand-based targeting (e.g., folate), and pharmacokinetic profiling in rodent models. Scalable production via microfluidics also warrants investigation.

Overall, F2 emerges as a strong candidate for cost-effective nanotherapeutic development, offering controlled, pH-responsive DOX delivery suitable for precision oncology.

Conclusion

The optimized DOX-loaded chitosan nanoparticles (F2) demonstrated ideal physicochemical properties—180 nm size, +35 mV zeta potential, and 87% encapsulation efficiency—making them excellent carriers for controlled and targeted drug delivery. Their strong pH-responsive biphasic release profile significantly improves upon free DOX by enhancing tumor-specific delivery while limiting off-target toxicity. The use of ionic gelation, a simple and scalable method, contributes to the feasibility of producing these nanoparticles for broader clinical applications. The release kinetics aligned well with the Higuchi model, supporting sustained exposure at the tumor site. Overall, this formulation shows strong potential for preclinical advancement, requiring further *in vivo* validation, safety studies, and targeted modifications for applications in breast cancer, sarcoma, and other solid tumors.

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