RESEARCH PAPER

FORMULATION, CHARACTERIZATION AND DRUG-RELEASE EVALUATION OF DOXORUBICIN-LOADED CHITOSAN NANOSPONGES

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ABSTRACT

Background: Doxorubicin hydrochloride is an established anthracycline anticancer agent whose pharmaceutical use is limited by non-selective distribution and dose-related toxicity. A chitosan–cyclodextrin carrier may improve drug association and provide controlled release without modifying the active molecule.

Objective: The study aimed to formulate, characterize and evaluate the drug-release behaviour of doxorubicin-loaded chitosan nanosponges prepared by ionic gelation.

Methods: Chitosan was dissolved in 1% v/v acetic acid, combined with β-cyclodextrin and doxorubicin hydrochloride, and ionically cross-linked by dropwise addition of sodium tripolyphosphate. The dispersion was stirred, probe-sonicated, centrifuged, washed, redispersed in 2% w/v mannitol and lyophilised. Preliminary batches were screened for appearance, separation, redispersibility and entrapment efficiency. The selected formulation contained 0.670% w/v chitosan, 1.500% w/v β-cyclodextrin and 0.100% w/v sodium tripolyphosphate. Particle size, polydispersity index, zeta potential, entrapment efficiency, FTIR compatibility, surface morphology, in vitro drug release, release kinetics and 30-day stability were evaluated.

Results: The selected formulation showed a particle size of 171.6 ± 3.8 nm, PDI of 0.189 ± 0.006, zeta potential of +24.9 ± 0.9 mV and entrapment efficiency of 80.42 ± 1.20%. FTIR retained the principal bands of the drug and excipients with broadening and small shifts, while SEM showed aggregated, irregular-to-nearly spherical dried particles with rough surface features. Drug release reached 22.48 ± 1.44% at 30 min, 58.64 ± 1.75% at 4 h and 87.86 ± 1.94% at 24 h. The Korsmeyer–Peppas model gave the best fit (R^2 = 0.989; n = 0.61), indicating anomalous transport. Refrigerated samples showed smaller physicochemical changes than room-temperature samples during 30 days of storage.

Conclusion: The chitosan–β-cyclodextrin nanosponge formulation provided nanoscale dimensions, a comparatively narrow size distribution, positive surface character, substantial doxorubicin entrapment and prolonged release, supporting its further development as a polymeric drug-delivery system.

Keywords: doxorubicin hydrochloride; chitosan; β-cyclodextrin; sodium tripolyphosphate; nanosponges; ionic gelation; controlled release

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INTRODUCTION

Doxorubicin hydrochloride is a broad-spectrum anthracycline used in the treatment of breast cancer, haematological malignancies, sarcomas and several other solid tumours. Its antineoplastic action involves intercalation between DNA base pairs, stabilisation of the topoisomerase-II cleavage complex and induction of oxidative and mitochondrial stress.[1] Despite its established efficacy, conventional doxorubicin therapy is constrained by non-selective exposure, myelosuppression, mucosal toxicity and cumulative cardiotoxicity. These limitations have encouraged formulation strategies that alter drug presentation, distribution and release while preserving the pharmacological activity of the parent compound.[1,2]

Nanocarrier systems do not automatically provide tumour selectivity, but they can improve the pharmaceutical handling of a drug, protect it from premature loss, modify its free-drug fraction and produce a more controlled temporal exposure.[2] Cyclodextrin-based nanosponges are porous or cross-linked networks that combine cyclodextrin inclusion domains with additional interstitial

spaces. A drug may associate with the internal cyclodextrin cavity, cross-linker-derived regions, hydrated pores or the external particle surface.[3,4] This multi-domain association is useful for amphiphilic molecules such as doxorubicin, which possesses both a planar aromatic anthracycline region and hydrophilic ionisable groups.

β-Cyclodextrin is a cyclic oligosaccharide containing seven glucopyranose units. Its outer surface is hydrophilic, whereas the internal cavity is relatively non-polar. The aromatic part of doxorubicin can partially associate with this cavity, while hydroxyl and amino groups may participate in hydrogen bonding with the surrounding matrix.[5-7] β-Cyclodextrin alone, however, does not provide a mechanically stable particulate matrix. Incorporation into a polymeric system can create a more structured carrier with additional control over drug retention and release.

Chitosan is a biodegradable cationic polysaccharide containing protonatable amino groups. In acidic medium, these amino groups become positively charged and can interact with multivalent phosphate ions. Sodium tripolyphosphate (TPP) is widely used to form chitosan particles by ionic gelation under mild aqueous conditions.[8-10] The process avoids harsh covalent cross-linkers and high temperatures, which is advantageous for light- and heat-sensitive compounds. Particle formation depends on chitosan concentration, TPP concentration, order of addition, local mixing, ionic strength and sonication. An inadequate polymer–cross-linker balance may produce weak particles or poor drug retention, whereas excessive cross-linking may promote aggregation and slow release.

Combining chitosan with β-cyclodextrin therefore offers complementary functions. Chitosan forms the hydrated cationic matrix and contributes surface charge, TPP creates ionic junctions, and β-cyclodextrin provides host-like domains for drug association. Previous chitosan–cyclodextrin carriers have shown that this combination can support doxorubicin loading and pH-dependent or sustained release.[6,7] Nevertheless, the behaviour of each formulation must be established experimentally because differences in material grade, composition and processing can substantially alter size, distribution, entrapment and release.

The present study was undertaken to formulate and evaluate doxorubicin-loaded chitosan nanosponges containing β-cyclodextrin and ionically cross-linked with sodium tripolyphosphate. The investigation included preformulation assessment, preliminary batch screening, preparation of the selected formulation, particle-size and surface-charge analysis, entrapment-efficiency determination, FTIR compatibility assessment, SEM examination, in vitro drug-release testing, release-kinetic modelling and short-term stability evaluation. The study was designed to determine whether the chitosan–β-cyclodextrin–TPP matrix could provide nanoscale dimensions, a comparatively narrow size distribution, positive surface character, satisfactory doxorubicin entrapment and prolonged release under the selected experimental conditions.

MATERIALS AND METHODS

Materials

Doxorubicin hydrochloride was obtained as a pharmaceutical-grade gift sample from Neon Laboratories Ltd., Navi Mumbai, India. Low-molecular-weight chitosan was procured from Sigma-Aldrich/Merck Life Science, India. β-Cyclodextrin was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Sodium tripolyphosphate was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Mannitol, glacial acetic acid, methanol, hydrochloric acid and buffer salts were of analytical or laboratory grade. Distilled water was used throughout. Doxorubicin and drug-containing samples were protected from direct light during weighing, preparation, analysis and storage.

Preformulation studies

Organoleptic and thermal observations

The colour, physical form, uniformity, visible contamination and lump formation of doxorubicin hydrochloride were examined visually. Thermal behaviour was studied by the capillary method. Because doxorubicin may decompose during heating, the temperature associated with darkening and decomposition was recorded rather than interpreted as a sharp melting point.

Solubility study

Apparent saturation solubility was determined in phosphate buffer pH 7.4 and methanol:0.1 N HCl. An excess of drug was added to each medium, mixed until equilibrium, centrifuged and filtered. The clear solution was diluted into the calibration range and analysed at 480 nm. Phosphate buffer was selected for release samples, whereas methanol:0.1 N HCl was used to extract and estimate free drug during entrapment-efficiency determination.

UV-visible spectrophotometry

A diluted doxorubicin solution was scanned between 400 and 600 nm against the corresponding blank, and the wavelength of maximum absorbance was identified. Calibration curves were prepared over 2–20 µg/mL in phosphate buffer pH 7.4 and methanol:0.1 N HCl. Concentration was plotted against absorbance, and linear-regression equations were calculated.

FTIR identification

The ATR-FTIR spectrum of pure doxorubicin hydrochloride was recorded between 4000 and 400 cm^{-1}. The principal hydroxyl/amino, aliphatic C–H, carbonyl and aromatic bands were used to support identity and were subsequently compared with the physical mixture and selected nanosponge formulation.

Preliminary formulation screening

Five exploratory batches were prepared before selection of the formal working range. Doxorubicin hydrochloride was maintained at 25 mg per 20 mL in these preliminary trials. Chitosan, β-

cyclodextrin, TPP and a small amount of poloxamer 188 were varied to identify regions associated with weak particle formation, excessive viscosity, aggregation or poor redispersion. Poloxamer 188 was used only during this feasibility stage and was excluded from the selected formulation so that the matrix consisted only of chitosan, β-cyclodextrin and TPP. Preliminary batches were assessed for appearance, sedimentation, centrifugation behaviour, redispersibility and entrapment efficiency.

Table 1: Composition and outcome of preliminary trial batches

Trial	Chitosan (%)	β-CD (%)	TPP (%)	Poloxamer 188 (%)	Entrapment efficiency (%)	Main observation	Decision
PT1	0.20	0.30	0.10	0.05	48.36 ± 1.42	Poor yield and weak particle formation	Rejected
PT2	0.30	0.40	0.15	0.08	61.84 ± 1.68	Fine opalescent dispersion; moderate entrapment	Range support
PT3	0.50	0.60	0.20	0.10	72.56 ± 1.35	Uniform dispersion; good separation and redispersion	Middle range
PT4	0.70	0.80	0.25	0.10	76.28 ± 1.52	Dense dispersion; higher size tendency	Upper range
PT5	0.90	0.90	0.30	0.12	78.14 ± 1.74	Viscous, aggregated and difficult to redisperse	Rejected

Preparation of the selected nanosponge formulation

Chitosan was dispersed in 1% v/v acetic acid and stirred until a clear or uniformly hydrated polymer solution was obtained. β-Cyclodextrin was dissolved separately in distilled water and added slowly to the chitosan solution under continuous stirring. Doxorubicin hydrochloride solution was incorporated into the polymer phase and mixed under light-protected conditions. Fresh aqueous TPP solution was then added dropwise at approximately 1000 rpm. Stirring was continued for 2 h to permit ionic interaction between protonated chitosan amino groups and phosphate groups of TPP. The dispersion was probe-sonicated at 30% amplitude for 3 min using a pulse cycle of 30 s on and 10 s off, with the sample maintained in an ice bath to limit temperature rise.[8-10]

The nanosponge dispersion was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was collected for estimation of unentrapped drug. The pellet was washed twice with distilled water, redispersed in 2% w/v mannitol, frozen at −20°C for 12 h and lyophilised for 24 h. Doxorubicin hydrochloride was maintained at 25 mg per 20 mL batch, consistent with the fixed drug level used during formulation screening. The selected matrix composition is presented in Table 2.

Table 2: Composition of the selected doxorubicin-loaded nanosponge formulation

Component	Selected level	Function in formulation
Chitosan	0.670% w/v	Cationic matrix-forming polymer
β-Cyclodextrin	1.500% w/v	Host-like drug-association domains
Sodium tripolyphosphate	0.100% w/v	Ionic cross-linking agent
Doxorubicin hydrochloride	25 mg per 20 mL batch	Anticancer drug payload
Mannitol	2% w/v before lyophilisation	Bulking and cryoprotective support

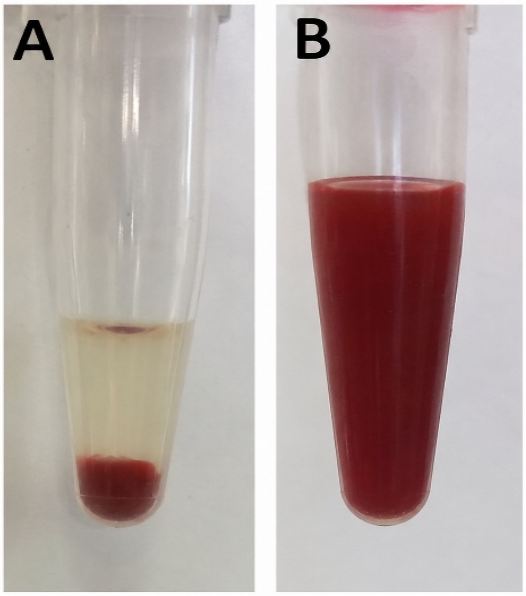

Figure 1: Visual appearance of the doxorubicin-loaded nanosponge system: (A) separated pellet after centrifugation and (B) uniformly redispersed drug-loaded nanosponge suspension.

Particle size, PDI and zeta potential

The nanosponge dispersion was diluted suitably with distilled water. Hydrodynamic particle size and PDI were measured by dynamic light scattering, and zeta potential was determined by electrophoretic light scattering using a Malvern Zetasizer Nano ZS90. Measurements were performed at 25°C using water as dispersant and were recorded in triplicate. Particle size was interpreted together with PDI because the mean diameter alone does not describe the breadth of the distribution.

Entrapment efficiency

A fixed volume of nanosponge dispersion was centrifuged at 15,000 × g for 20 min at 4°C. The free doxorubicin present in the supernatant was analysed at 480 nm after suitable dilution in methanol:0.1 N HCl. Entrapment efficiency was calculated according to the following equation: Entrapment efficiency (%) = [(total drug added − free drug in supernatant)/total drug added] × 100. The experiment was performed in triplicate.

FTIR compatibility study

ATR-FTIR spectra of doxorubicin hydrochloride, chitosan, β-cyclodextrin, sodium TPP, their physical mixture and the selected lyophilised nanosponge formulation were recorded over 4000–400 cm^{-1}. The comparison focused on retention, broadening, attenuation and small positional shifts of characteristic bands. FTIR was used to assess compatibility and possible physical interaction; minor shifts alone were not interpreted as proof of new covalent-bond formation.

Scanning electron microscopy

Lyophilised blank and doxorubicin-loaded nanosponge powders were mounted on aluminium stubs using conductive adhesive, coated with a thin conductive layer and examined by scanning electron microscopy at suitable magnifications. SEM was used to describe dried-state shape, surface roughness, porosity and aggregation. Dimensions observed in dried SEM images were not equated with hydrodynamic diameters obtained by light scattering.

In vitro drug release

Drug release was studied by a dialysis-membrane diffusion method in phosphate buffer pH 7.4 at 37 ± 0.5°C under continuous stirring. Nanosponge dispersion equivalent to a fixed amount of doxorubicin was placed inside a pre-soaked dialysis membrane. Samples were collected at 5, 15 and 30 min and at 1, 2, 4, 8 and 24 h. Each withdrawn aliquot was replaced by an equal volume of fresh medium maintained at the same temperature. Doxorubicin was quantified at 480 nm, and cumulative percentage release was calculated after correction for sampling.

Release kinetic analysis

The release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The model with the highest coefficient of determination was considered the best mathematical fit. The Korsmeyer–Peppas exponent was used to support a descriptive interpretation of diffusion and matrix-relaxation contributions. These models were treated as empirical descriptions and not as independent proof of a molecular release mechanism.[12]

Short-term stability study

The lyophilised formulation was stored in tightly closed, light-resistant containers at 4 ± 2°C and 25

± 2°C. Samples were evaluated at 0, 15 and 30 days for physical appearance, redispersibility, particle size, PDI, zeta potential, entrapment efficiency and 24 h drug release. The study was intended to detect early physical instability and storage-condition sensitivity; it was not designed to establish a formal shelf life.[13-15]

Statistical analysis

Quantitative results are expressed as mean ± standard deviation (n = 3). Stability data were evaluated using two-way analysis of variance with storage condition and time as factors, followed by Tukey multiple-comparison testing. A probability value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preformulation characteristics of doxorubicin hydrochloride

Doxorubicin hydrochloride appeared as a fine orange-red to reddish crystalline powder with no unusual odour, visible foreign matter or marked lump formation. The colour and physical appearance were consistent with the expected anthracycline drug. Slight hygroscopicity required rapid weighing and storage in a tightly closed, light-resistant container.

The drug did not show a sharp melting transition. Darkening followed by decomposition was observed at 206.1 ± 2.2°C, which was close to the expected decomposition behaviour around 205°C. This result was considered supportive of identity but was not treated as a purity test because decomposition can be influenced by heating rate, sample packing and moisture content.

Apparent saturation solubility was 12.86 ± 0.52 mg/mL in phosphate buffer pH 7.4 and 16.42 ± 0.58 mg/mL in methanol:0.1 N HCl. The higher measured solubility in the acidic methanolic medium supported its use for extraction of unentrapped drug. Solubility in phosphate buffer pH 7.4 was adequate for spectrophotometric measurement of the release samples within the selected concentration range.

Table 3: Preformulation results of doxorubicin hydrochloride

Parameter	Observation/result	Purpose	Interpretation
Appearance	Orange-red to reddish crystalline powder	Identity and handling	Expected appearance; slight hygroscopicity
Thermal observation	206.1 ± 2.2°C with darkening/decomposition	Basic identity support	Thermal decomposition rather than sharp melting
Solubility in pH 7.4 buffer	12.86 ± 0.52 mg/mL	Release analysis	Suitable analytical medium
Solubility in methanol:0.1 N HCl	16.42 ± 0.58 mg/mL	Free-drug extraction	Improved extraction medium
λmax	480 nm	Quantitative UV analysis	Visible-region absorbance of anthracycline chromophore

UV-visible analytical performance

The visible spectrum showed maximum absorbance at 480 nm. Calibration was linear between 2 and 20 µg/mL in both media. In phosphate buffer pH 7.4, the regression equation was $y = 0.04164x + 0.00474$ with $R^2 = 0.99933$. In methanol:0.1 N HCl, the regression equation was $y = 0.04312x + 0.00396$ with $R^2 = 0.99918$. The high coefficients of determination demonstrated a strong concentration–absorbance relationship over the working range and supported use of the calibration equations for release and unentrapped-drug analysis.

Table 4: UV-visible calibration parameters for doxorubicin hydrochloride

Medium	λmax	Range	Regression equation	R^2	Application
Phosphate buffer pH 7.4	480 nm	2–20 µg/mL	$y = 0.04164x + 0.00474$	0.99933	Release samples

Medium	λmax	Range	Regression equation	R^2	Application
Methanol:0.1 N HCl	480 nm	2–20 µg/mL	y = 0.04312 x + 0.00396	0.99918	Free-drug extraction

Outcome of preliminary screening

The preliminary batches demonstrated that formulation performance depended on both component concentration and practical process behaviour. PT1 showed weak nanosponge formation and low entrapment efficiency, suggesting that the available polymeric matrix was insufficient for effective particle recovery and drug retention. PT2 improved opalescence and entrapment but remained only moderately satisfactory. PT3 produced a uniform dispersion, acceptable centrifugation behaviour and good redispersibility, identifying a workable middle region. PT4 increased entrapment but produced a denser dispersion with a tendency towards larger particles. PT5 showed the highest preliminary entrapment efficiency but was highly viscous, aggregated and difficult to redisperse. Thus, the formulation with the numerically highest entrapment was not considered the most practical. This screening emphasised that drug retention must be balanced against particle handling, uniformity and recoverability.

Appearance and recovery of the selected formulation

After ionic gelation, the drug-loaded system formed a uniform red nanosponge dispersion. Refrigerated centrifugation produced a distinct drug-containing pellet, while washing removed unentrapped material. Redispersion in mannitol solution restored a relatively uniform suspension before freezing and lyophilisation. The recovered dry powder remained redispersible, indicating that the selected 2% w/v mannitol level provided useful bulking and cryoprotective support during the short laboratory process. The appearance did not establish chemical stability, but it provided an initial indication that the particles could be separated, dried and redispersed without immediate hard-cake formation.

Particle size and polydispersity

The selected formulation showed a Z-average particle size of 171.6 ± 3.8 nm and a PDI of 0.189 ± 0.006. The instrument report showed a single dominant intensity distribution near the nanoscale mean and classified the result quality as good (Figure 2). A PDI below 0.20 indicated a comparatively narrow distribution under the measurement conditions. The observed particle size reflected the combined influence of chitosan concentration, ionic cross-linking, controlled TPP addition and probe sonication. Similar chitosan–TPP systems are known to be highly sensitive to cross-linking stoichiometry and mixing conditions.[8-10]

The hydrodynamic diameter includes the hydrated polymer layer and any associated solvent at the slipping boundary. It should therefore not be expected to match the apparent dimensions of dried particles in SEM. The particle-size result also does not by itself establish long-term colloidal stability, which must be interpreted together with PDI, zeta potential and storage behaviour.

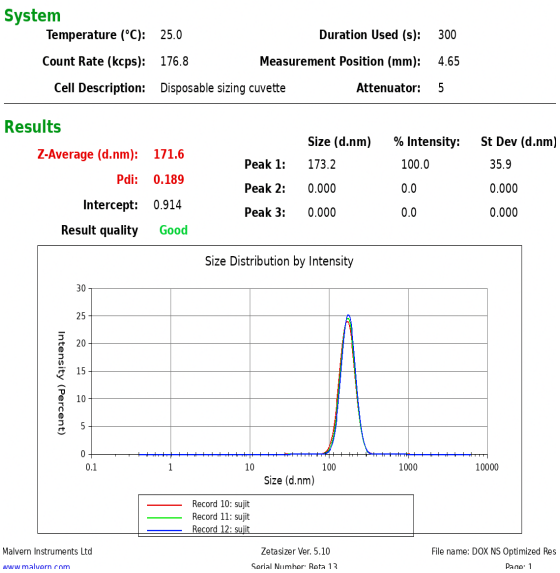

Figure 2: Dynamic light-scattering report of the selected doxorubicin-loaded nanosponge formulation showing a Z-average particle size of 171.6 nm and PDI of 0.189.

Zeta potential

The mean zeta potential was +24.9 ± 0.9 mV, and the instrument report showed one dominant positive distribution (Figure 3). The positive surface character was expected because protonated chitosan amino groups remained accessible at the particle surface. TPP neutralises a fraction of these amino groups during ionic junction formation, but the selected low cross-linker level preserved a net positive potential. A value near +25 mV can contribute to electrostatic repulsion and may support short-term dispersion stability, although zeta potential is strongly dependent on pH, ionic strength and dilution medium and cannot independently predict shelf life.

The positive surface may also favour interaction with negatively charged biological interfaces. However, zeta potential alone cannot establish cellular uptake or membrane interaction, and the present result was therefore interpreted principally as evidence of the chitosan-rich particle surface and its contribution to short-term dispersion behaviour.

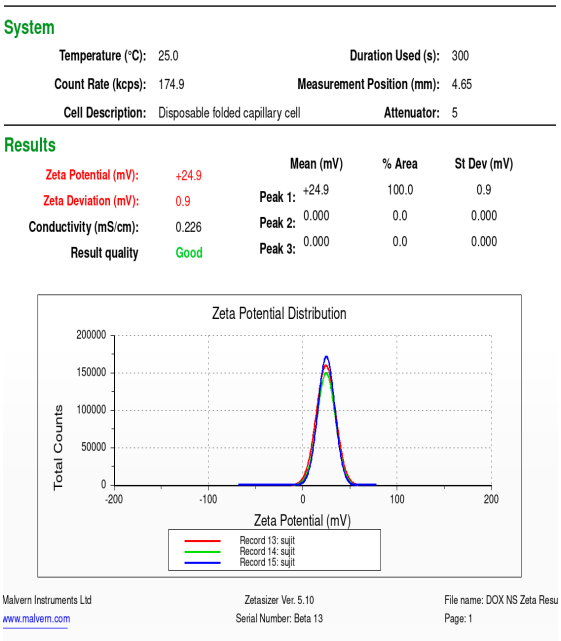

Figure 3: Zeta-potential distribution report of the selected doxorubicin-loaded nanosponge formulation showing a mean value of +24.9 mV.

Entrapment efficiency and overall physicochemical profile

Entrapment efficiency was 80.42 ± 1.20%, indicating that most of the initially incorporated doxorubicin remained associated with the particles after centrifugation and washing. Retention was likely produced by a combination of physical confinement within the chitosan–TPP network, hydrogen bonding and partial association of the anthracycline region with β-cyclodextrin domains.[6,7] Because doxorubicin and chitosan both contain protonatable amino groups, the retention mechanism should not be described as simple direct electrostatic attraction between the drug and polymer.

Entrapment efficiency represents the percentage of the added drug retained by the carrier after separation of the unentrapped fraction. It does not directly represent drug loading per unit mass of dried formulation. Determination of production yield and gravimetric drug loading would provide additional information for dose calculation, scale-up and comparison with other carrier systems.

Table 5: Core physicochemical characteristics of the selected formulation

Attribute	Result	Desired basic characteristic	Interpretation
Particle size	171.6 ± 3.8 nm	Nanoscale size	Hydrated nanosponge dispersion
PDI	0.189 ± 0.006	Low distribution breadth	Comparatively uniform population
Zeta potential	+24.9 ± 0.9 mV	Positive chitosan-rich surface	Moderate electrostatic contribution
Entrapment efficiency	80.42 ± 1.20%	High drug retention	Substantial association after washing
24 h release	87.86 ± 1.94%	Prolonged release	Near-complete release over 24 h

FTIR compatibility and matrix association

Pure doxorubicin hydrochloride showed characteristic bands in the broad O–H/N–H region near 3440 cm^{-1}, aliphatic C–H stretching near 2926 cm^{-1}, carbonyl stretching near 1728 cm^{-1} and aromatic vibrations near 1615 cm^{-1}. Chitosan showed a broad hydroxyl/amino region and amide-associated bands, β-cyclodextrin showed broad hydroxyl and strong C–O/C–O–C vibrations, and sodium TPP showed phosphate-region bands. The physical mixture retained the principal features of the components.

The selected formulation retained the major drug- and excipient-associated regions, but broadening, attenuation and small positional shifts were present. This pattern was consistent with physical association, hydrogen bonding and ionic interaction within the hydrated chitosan–β-cyclodextrin–TPP matrix. No new dominant band suggesting an obvious incompatible chemical transformation was observed. Similar retention-with-broadening patterns have been reported in related doxorubicin-loaded chitosan–cyclodextrin carriers.[7] FTIR alone cannot establish complete inclusion of doxorubicin inside β-cyclodextrin or quantify the fraction of drug in different matrix domains.

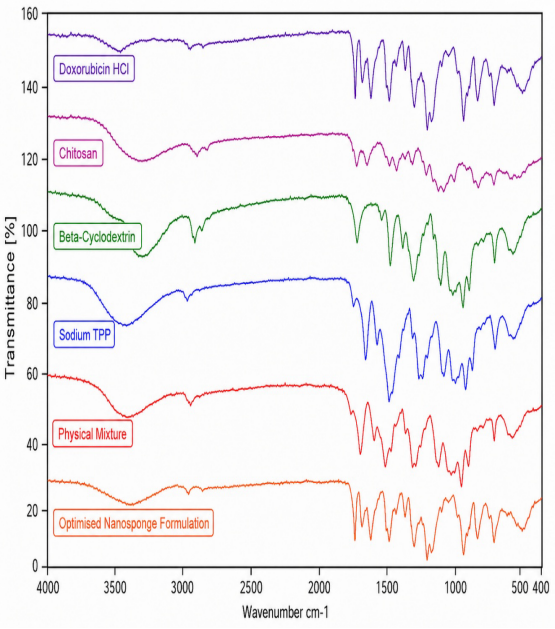

Figure 4: FTIR spectra of doxorubicin hydrochloride, chitosan, β-cyclodextrin, sodium TPP, physical mixture and selected doxorubicin-loaded nanosponge formulation.

Surface morphology

SEM showed clustered, irregular-to-nearly spherical dried particles with rough and porous surface features (Figure 5). Blank particles formed aggregated nanosponge-like masses, whereas the drug-loaded material appeared comparatively compact but retained surface depressions and textural irregularity. The aggregation was expected because lyophilised particles were brought into close contact during freezing and drying and were subsequently mounted and conductively coated for SEM.

The micrographs supported formation of a particulate dried matrix rather than a smooth continuous film. SEM was used only to describe external dried-state morphology. Internal porosity, drug distribution within individual particles and hydrodynamic diameter in dispersion would require complementary analytical techniques and were not inferred from these images.

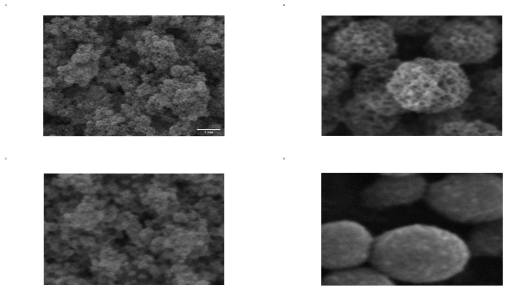

Figure 5: Representative SEM images of blank nanosponges (upper panels) and selected doxorubicin-loaded chitosan–β-cyclodextrin nanosponges (lower panels) at low and higher magnifications.

In vitro drug-release profile

The selected formulation released 9.84 ± 1.10% of doxorubicin within 5 min, 15.62 ± 1.26% at 15 min and 22.48 ± 1.44% at 30 min. The limited early release suggested that only a comparatively small fraction of drug was weakly associated with the outer matrix or readily accessible aqueous channels. Release then increased gradually to 31.36 ± 1.58% at 1 h, 43.82 ± 1.67% at 2 h, 58.64 ± 1.75% at 4 h and 72.48 ± 1.86% at 8 h. At 24 h, cumulative release reached 87.86 ± 1.94%.

The gradual release phase was consistent with diffusion through the hydrated polymer–cyclodextrin network and progressive relaxation of the ionically cross-linked matrix. β-Cyclodextrin-associated drug may require dissociation before diffusion, while TPP-mediated junctions increase the path length through the polymer network. The incomplete release at 24 h may reflect residual strong association, matrix retention or dialysis-related transport limitations. Because the method measures combined movement through the formulation and the dialysis membrane, it should be interpreted as an in vitro comparative release test rather than a direct simulation of in vivo drug liberation.

Table 6: In vitro release of doxorubicin from the selected nanosponge formulation

Time	Cumulative release (%)	Interpretive phase
5 min	9.84 ± 1.10	Initial surface-accessible release
15 min	15.62 ± 1.26	Early release
30 min	22.48 ± 1.44	Limited initial burst
1 h	31.36 ± 1.58	Transition to matrix-controlled phase
2 h	43.82 ± 1.67	Progressive diffusion
4 h	58.64 ± 1.75	Sustained matrix release

Time	Cumulative release (%)	Interpretive phase
8 h	72.48 ± 1.86	Continued diffusion/relaxation
24 h	87.86 ± 1.94	Near-complete prolonged release

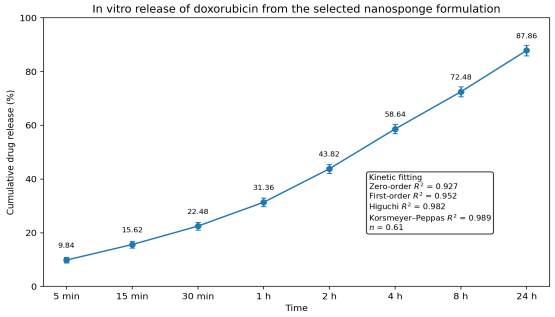

Figure 6: In vitro release profile of doxorubicin from the selected nanosponge formulation in phosphate buffer pH 7.4. Values are mean ± SD (n = 3).

Release kinetic analysis

The zero-order, first-order, Higuchi and Korsmeyer–Peppas R² values were 0.927, 0.952, 0.982 and 0.989, respectively. The Korsmeyer–Peppas model therefore provided the best mathematical fit, followed closely by the Higuchi model. The release exponent was n = 0.61, which falls within the anomalous or non-Fickian range for the empirical geometry applied in the analysis. The combined result suggested that release was not governed by a single ideal diffusion process. Instead, movement of the drug through hydrated pathways occurred together with polymer relaxation or rearrangement.

The relatively high Higuchi value supported an important diffusion contribution, whereas the improved Korsmeyer–Peppas fit indicated that polymer behaviour also influenced the profile. These model fits are descriptive and should not be interpreted as conclusive proof of microscopic release mechanisms. Additional studies at different pH values, ionic strengths and membrane conditions would be required to separate drug–cyclodextrin dissociation, matrix diffusion and membrane transport.

Table 7: Release kinetic parameters of the selected formulation

Model	Transformation used	R²	Interpretation
Zero-order	Cumulative release versus time	0.927	Lower fit; release not concentration-independent
First-order	Log drug remaining versus time	0.952	Improved concentration-dependent description
Higuchi	Cumulative release versus square root of time	0.982	Strong diffusion-related component
Korsmeyer–Peppas	Log fraction released versus log time	0.989; n = 0.61	Best fit; anomalous diffusion and relaxation

Short-term stability

No marked colour change, visible moisture uptake or hard-cake formation was observed during refrigerated storage. The powder remained redispersible. At 4 ± 2°C, particle size increased from 171.6 ± 3.8 nm at day 0 to 173.4 ± 4.1 nm at day 15 and 176.2 ± 4.6 nm at day 30. PDI increased modestly from 0.189 ± 0.006 to 0.201 ± 0.008, while zeta potential decreased from +24.9 ± 0.9 to +23.8 ± 0.9 mV. Entrapment efficiency and 24 h release remained 78.94 ± 1.32% and 86.28 ± 1.88%, respectively, at day 30.

Room-temperature storage produced larger changes. Particle size increased to 184.8 ± 5.2 nm and PDI to 0.219 ± 0.010 by day 30. Zeta potential decreased to +22.4 ± 1.1 mV, entrapment efficiency to 76.82 ± 1.44% and 24 h release to 84.12 ± 2.06%. The simultaneous increase in size and distribution breadth, together with reduced positive potential and drug retention, suggested gradual aggregation, surface rearrangement or drug leakage. These changes remained moderate during the 30-day period but were consistently greater at 25 ± 2°C.

The results indicated a refrigerated short-term storage preference. This agrees with reports that chitosan–TPP particles can undergo time- and temperature-dependent changes in swelling, aggregation and ionic-network organisation.[13-15] The study did not include chemical degradation

products, moisture content, sterility, photostability or long-term accelerated conditions. A formal shelf-life claim would therefore be inappropriate.

Condition	Day	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	24 h release (%)
4 ± 2°C	0	171.6 ± 3.8	0.189 ± 0.006	+24.9 ± 0.9	80.42 ± 1.20	87.86 ± 1.94
4 ± 2°C	15	173.4 ± 4.1	0.194 ± 0.007	+24.5 ± 0.8	79.86 ± 1.26	87.14 ± 1.82
4 ± 2°C	30	176.2 ± 4.6	0.201 ± 0.008	+23.8 ± 0.9	78.94 ± 1.32	86.28 ± 1.88

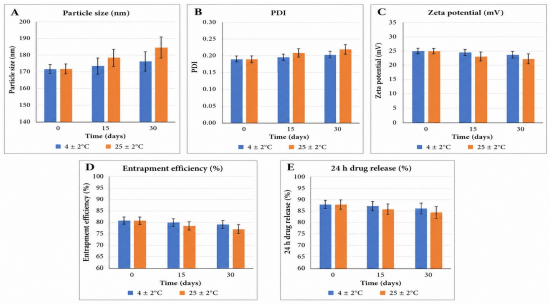

Figure 7: Stability profile of the selected doxorubicin-loaded nanosponge formulation stored at 4 ± 2°C and 25 ± 2°C for 30 days.

Overall pharmaceutical interpretation

The selected composition produced a balanced profile rather than maximising any single parameter. Higher-polymer preliminary trials improved entrapment but also increased viscosity and aggregation, whereas the selected formulation maintained nanoscale dimensions, low PDI, positive surface character and approximately 80% entrapment. The release profile avoided a large initial burst and provided gradual release over 24 h. This balance is pharmaceutically more meaningful than selection based only on entrapment efficiency.

The findings support formation of an ionically cross-linked chitosan matrix containing β-cyclodextrin-associated domains. The positive zeta potential and FTIR shifts were consistent with matrix formation, while SEM confirmed a particulate dried product. However, the data do not establish tumour targeting, improved pharmacokinetics, reduced cardiotoxicity or clinical superiority. Those outcomes require separate biological and in vivo evidence and should not be inferred from particle size or controlled release alone.

Table 8: Short-term stability of the selected doxorubicin-loaded nanosponge formulation

Condition	Day	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	24 h release (%)
25 ± 2°C	0	171.6 ± 3.8	0.189 ± 0.006	+24.9 ± 0.9	80.42 ± 1.20	87.86 ± 1.94
25 ± 2°C	15	178.5 ± 4.8	0.208 ± 0.009	+23.2 ± 1.0	78.26 ± 1.38	85.64 ± 1.96
25 ± 2°C	30	184.8 ± 5.2	0.219 ± 0.010	+22.4 ± 1.1	76.82 ± 1.44	84.12 ± 2.06

LIMITATIONS

The study was limited to in vitro formulation and physicochemical evaluation. Drug loading per unit mass, production yield, residual moisture, sterility and chemical-degradation products were not determined. The UV-visible method demonstrated linearity over the working range but was not evaluated as a complete stability-indicating analytical method. Dialysis-based release represents combined transport through the hydrated carrier and membrane and may not reproduce in vivo drug liberation. The 30-day study provides only short-term stability information and cannot support shelf-life assignment. Long-term stability, pharmacokinetic, biodistribution, safety and efficacy investigations are therefore required before translational conclusions can be drawn.

CONCLUSION

Doxorubicin-loaded chitosan–β-cyclodextrin nanosponges were successfully prepared by ionic gelation with sodium tripolyphosphate and

recovered as a redispersible lyophilised powder. Preliminary screening demonstrated that formulation selection should consider handling and redispersibility in addition to entrapment efficiency because excessive polymer and cross-linker levels increased viscosity and aggregation. The selected formulation containing 0.670% w/v chitosan, 1.500% w/v β-cyclodextrin and 0.100% w/v TPP showed a mean particle size of 171.6 nm, PDI below 0.20, positive zeta potential near +25 mV and entrapment efficiency of approximately 80%. FTIR and SEM findings were consistent with drug association within a particulate chitosan–cyclodextrin matrix. Drug release was gradual over 24 h and was best described by the Korsmeyer–Peppas model with an anomalous transport exponent. Refrigerated storage preserved the physicochemical profile more effectively than room-temperature storage during the 30-day study. These results support further pharmaceutical development of the system through drug-loading and yield determination, analytical-method validation, long-term stability testing and appropriately designed biological investigations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable to the formulation and physicochemical work reported in this manuscript.

DATA AVAILABILITY

The formulation data supporting this manuscript are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

Dr. Sonal Solanki: supervision, methodology review, interpretation of results, manuscript review and editing. Sujit Bendugade: investigation, formulation preparation, data collection, analysis and original manuscript drafting. Ashok Dalimbe: methodology support, resources, validation and manuscript review. Dr. Nikunj Solanki: conceptualisation, study design, supervision, interpretation, project administration, manuscript review and correspondence. The specific CRediT contributions of Dr. K. Kombaiah, Dr. Papiya Sinha, Harsh Goswami, Diksha Rana, Dr. Manpreet Kaur, Mr. Rounak Jaiswal, Dr. Sunil Nirmal and Shreya Shinde should be confirmed by the corresponding author before submission. All listed authors should review and approve the final manuscript.

REFERENCES

1. Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem. 2009;16(25):3267-3285.
2. Min Y, Caster JM, Eblan MJ, Wang AZ. Clinical translation of nanomedicine. Chem Rev. 2015;115(19):11147-11190.
3. Trotta F, Zanetti M, Cavalli R. Cyclodextrin-based nanosponges as drug carriers. Beilstein J Org Chem. 2012;8:2091-2099.
4. Swaminathan S, Cavalli R, Trotta F. Cyclodextrin-based nanosponges: a versatile platform for cancer nanotherapeutics development. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2016;8(4):579-601.
5. Szejtli J. Medicinal applications of cyclodextrins. Med Res Rev. 1994;14(3):353-386.
6. Lu L, Shao XH, Jiao Y, et al. Synthesis of chitosan-graft-β-cyclodextrin for improving the loading and release of doxorubicin in nanoparticles. J Appl Polym Sci. 2014;131(21):41034.
7. Mi Y, Zhang J, Tan W, et al. Preparation of doxorubicin-loaded carboxymethyl-β-cyclodextrin/chitosan nanoparticles with antioxidant, antitumor activities and pH-sensitive release. Mar Drugs. 2022;20(4):278.
8. Masarudin MJ, Cutts SM, Evison BJ, Phillips DR, Pigram PJ. Factors determining the stability, size distribution and cellular accumulation of small monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to passive encapsulation of doxorubicin. Nanotechnol Sci Appl. 2015;8:67-80.
9. Sreekumar S, Goycoolea FM, Moerschbacher BM, Rivera-Rodriguez GR. Parameters influencing the size of chitosan-TPP nano- and microparticles. Sci Rep. 2018;8:4695.
10. Koukaras EN, Papadimitriou SA, Bikiaris DN, Froudakis GE. Insight on the formation of chitosan nanoparticles through ionotropic gelation with tripolyphosphate. Mol Pharm. 2012;9(10):2856-2862.
11. Huang Y, Lapitsky Y. Monovalent salt enhances colloidal stability during the formation of chitosan/tripolyphosphate microgels. Langmuir. 2011;27(17):10392-10399.
12. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chem Rev. 2016;116(4):2602-2663.
13. Morris GA, Castile J, Smith AM, Adams GG, Harding SE. The effect of prolonged storage

at different temperatures on the particle size distribution of tripolyphosphate-chitosan nanoparticles. Carbohydr Polym. 2011;84(4):1430-1434.
14. Jonassen H, Kjøniksen AL, Hiorth M. Stability of chitosan nanoparticles cross-linked with tripolyphosphate. Biomacromolecules. 2012;13(11):3747-3756.
15. Huang Y, Cai Y, Lapitsky Y. Factors affecting the stability of chitosan/tripolyphosphate micro- and nanogels: resolving opposing findings. J Mater Chem B. 2015;3(29):5957-5970.