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DEVELOPMENT AND BIOCHEMICAL EVALUATION OF A NOVEL BIOPOLYMER-BASED DRUG DELIVERY SYSTEM FOR ENHANCED THERAPEUTIC EFFICACY

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ABSTRACT

Biopolymer-based nanocarriers offer a promising strategy for improving drug stability, controlled release, and intracellular therapeutic delivery. This study developed and computationally evaluated a chitosan-alginate nanoparticle system for doxorubicin delivery using a dataset encompassing formulation, drug-release, biochemical efficacy, and stability parameters. Among 120 candidate formulations, F110 achieved the highest composite desirability (0.7879), with a particle size of 209 nm, polydispersity index of 0.252, encapsulation efficiency of 86.5%, drug loading of 19.1%, and production yield of 96.0%. Multivariable analysis identified drug-to-polymer ratio, chitosan, and alginate as major determinants of formulation performance. Nanoparticle incorporation prolonged the time to 50% drug release from 0.650 h for free doxorubicin to 3.301 h at pH 5.5 and 6.632 h at pH 7.4. Full release profiles followed first-order kinetics, while early nanoparticle release showed anomalous/non-Fickian behavior. Loaded nanoparticles demonstrated enhanced cellular uptake, reactive oxygen species generation, caspase-3 activity, and greater reductions in cell viability at selected doses. Refrigerated storage better preserved physicochemical stability over 90 days. Overall, the optimized system demonstrated favorable formulation, controlled-release, biochemical, and stability characteristics, supporting further experimental validation for advanced drug-delivery applications.

KEYWORDS: Biopolymer nanoparticles, Chitosan-alginate, Doxorubicin, Controlled drug release, Therapeutic efficacy.

1. INTRODUCTION

Drug delivery systems have emerged as an important part of the pharmacotherapy of today, as the effectiveness of therapy is not only determined by the therapeutic activity of the drug, but also the ability to deliver the drug to the proper site of action in the body. Poor solubility, degradation, fast clearance, undesirable distribution, and systemic toxicity in conventional administration is a need for the development and use of carrier for better stability, bioavailability, and controlled exposure to the target site (Jain, 2019). While great strides have been made in the delivery of nanotechnology, there are still difficulties associated with reproducibility, biocompatibility, manufacturing and clinical translation (Ezike et al., 2023). While nanocarriers are able to tackle specific delivery challenges, they require chemistry and interactions with complex biological environments to perform effectively (Reinholz et al., 2018).

Natural Biopolymer can be used as a foundation for advanced drug delivery systems due to their biodegradability, biocompatibility, versatility in structure and potential for chemical or physical manipulation. Therapeutic molecules can be encapsulated within biopolymer-based carriers to prevent degradation, and controlled release can be achieved by tuning the polymer network (Fazal et al., 2023). The Versatile Physicochemical Properties have thus paved the way for the formulation of nanoparticles, hydrogels, films, and composite systems for pharmaceutical applications (Opriș et al., 2024). Chitosan is one of these materials of interest that can be used to efficiently form nanoparticles and incorporate a drug due to its cationic nature, functional amino groups, mucoadhesive properties, and ionic crosslinking ability (Desai et al., 2023). The potential of chitosan nanoparticles for anticancer drug delivery is also remarkable, but the biological performance can be modified depending on the formulation, such as the size and surface charge of the particles and the percentage of drug encapsulation and stability (Sachdeva et al., 2023).

The synergistic use of chitosan and alginate is a rational approach to the creation of polyelectrolyte nanoparticle matrices. Chitosan is positively charged whereas alginate is negatively charged, allowing for the formation of biocompatible carriers under relatively mild conditions, and controlling the drug loading, swelling, stability, and release behaviour (Wathoni et al., 2024). These properties are important for delivery of doxorubicin, in which controlled intracellular availability can influence the therapeutic efficacy and minimize early exposure. Furthermore, in advanced delivery design, it is becoming more significant to be able to respond to the environment to adjust the delivery of the payload. The pH responsive behavior is important in certain situations where the physiological / acidic cellular environment is very different. Recent work with

stimuli-responsive systems shows that the architecture of the polymer can control the release of active material in response to external stimuli (Shi et al., 2024), and more general research in stimuli-responsive delivery highlights the need to match the composition of the carrier with its responsiveness to the stimulus (Hu et al., 2023).
These improvements, however, do not always lead to the proper biochemical evaluation of the formulation. Formulation–property–release–response relationship must be defined simultaneously considering particle properties, encapsulation efficiency, drug loading, drug release, cellular uptake and biologically relevant endpoints. An integrated evaluation is critical for the identification of formulation properties that are at the heart of the delivery performance and for establishing mechanistic relationships between the properties of the carrier, its type of drug availability, and outcome of therapeutic response. Thus, the aim of this study was to design and optimize a chitosan–alginate nanoparticle system for doxorubicin delivery, to characterize the physic-chemical properties, the pH dependent release and stability of the system, and to assess the therapeutic performance by means of assays of cellular uptake, cell viability, generation of reactive oxygen species, and activity of caspase-3. This integrated framework has been developed for evaluating whether a controlled drug release could be achieved with optimized delivery using biopolymers and if cellular and biochemical responses could be improved.

Research Objectives

1.To develop and optimize a chitosan–alginate nanoparticle system for efficient doxorubicin delivery.
2.To evaluate the physicochemical properties, pH-responsive drug release, and stability of the optimized formulation.
3.To assess its therapeutic and biochemical efficacy through cellular uptake, cell viability, ROS generation, and caspase-3 activity.

2. Materials and Methods

2.1 Study Design and Dataset Generation

To study a chitosan-alginate nanoparticle system as a drug carrier for doxorubicin, a simulation-based quantitative design was used. Experimentally plausible ranges of formulation and processing variables were used to generate a dataset of 120 formulations. Physicochemical characteristics, drug-release profiles, biochemical responses and stability parameters were included in the dataset. All the observations were assumed to be simulated and not from laboratory experiments.

2.2 Formulation Development and Optimization

Formulation variables were chitosan concentration, alginate concentration, drug to polymer ratio, crosslinker concentration, formulation pH, stirring speed and stirring time. These optimization responses were determined as particle size, polydispersity index, zeta potential, encapsulation efficiency, drug loading capacity, yield and swelling ratio. These responses were combined using a composite desirability approach. The drug delivery system with the greatest overall desirability was chosen as the optimized system for delivering the drug.

2.3 Physicochemical Characterization and Drug-Release Evaluation

The optimized formulation was characterised in terms of Particle size, PDI, zeta potential, EE, DL, Y, and Swelling behavior. The cumulative release of doxorubicin was quantified after specific periods under physiological conditions (pH 7.4) and under acidic conditions (pH 5.5). Where applicable, release profiles were compared to free-drug controls. Release behavior was investigated by fitting the drug-release data to zero-order, first-order, Higuchi and Korsmeyer–Peppas models.

2.4 Biochemical Efficacy and Stability Assessment

Therapeutic efficacy was evaluated by cellular uptake of the drug, cell viability, generation of ROS, and caspase-3 activity. The responses to nanoparticle loaded doxorubicin were compared to free doxorubicin and relevant controls at predetermined concentrations. The particle size, PDI, EE and DR were also selected as stability indicators to assess their formulation stability at 4°C and 25°C. Temporal changes were studied to identify the formulation condition which resulted in the highest stability.

2.5 Statistical Analysis

Descriptive statistics (mean ± SD) were used for continuous variables. Correlation and regression analyses were performed to determine the relationships between the formulation parameters and response variables. Differences between experimental conditions were evaluated using oneway ANOVA or two-way ANOVA with appropriate post-hoc comparisons. The goodness of fit of the release models was compared with suitable regression statistics and the significance level was set at $p < 0.05$.

3. RESULTS

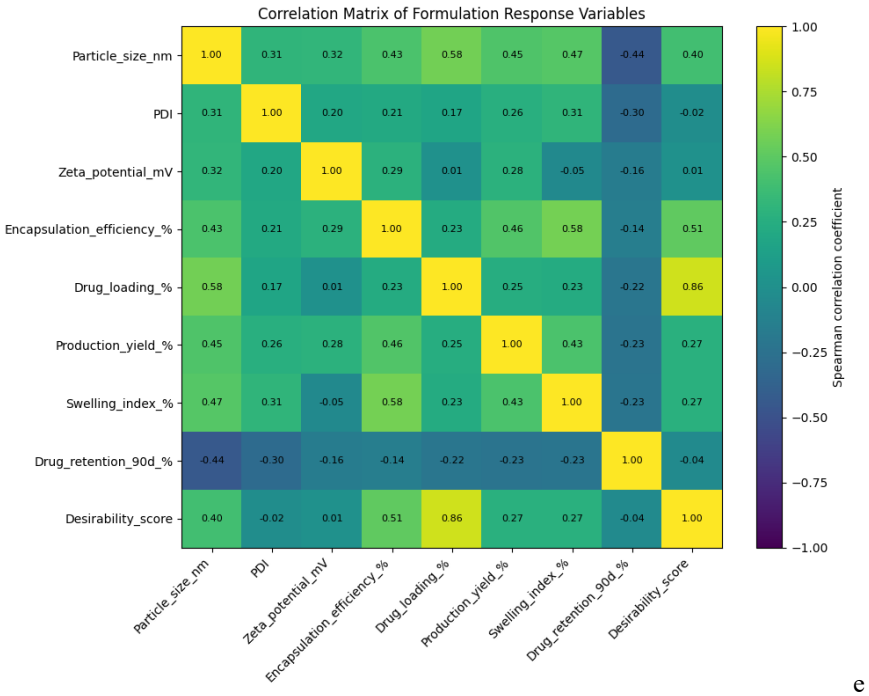
3.1 Formulation Development and Optimization

All 120 of the formulations were tested for seven formulation and processing variables. Particle size ranged from 110.8 to 229.8 nm, encapsulation efficiency from 57.4% to 94.7%, drug loading from 3.2% to 19.1%, and composite desirability from 0.5957 to 0.7879. Based on the data presented in Table 1, formulation F110 was found to be the best formulation and was considered the optimum formulation. The 1.94% w/v chitosan, 1.38% w/v alginate, drug-to-polymer ratio (DPR) 0.253 and 0.283% w/v crosslinker was present in it as demonstrated in figure 1.
The drug-to-polymer ratio was the major factor for drug loading and composite desirability in the multivariable analysis. Higher stirring speed showed an inverse correlation with particle size and increased concentration of chitosan and alginate

significantly affected the EE, PDI, drug loading and particle size. As seen in figure 2, the particle-size model accounted for 85.35% of variance while the drug-loading model accounted for 92.68% of variance.

Table 1. Optimized formulation characteristics and major formulation predictors

Parameter	Optimized value / statistical result
Formulation ID	F110
Chitosan (% w/v)	1.94
Alginate (% w/v)	1.38
Drug-to-polymer ratio	0.253
Crosslinker (% w/v)	0.283
Preparation pH	5.94
Stirring speed (rpm)	800
Stirring time (min)	30
Composite desirability	0.7879
Particle-size model R^2	0.8535
Drug-loading model R^2	0.9268
Drug/polymer ratio → drug loading, β	0.9159
Chitosan → particle size, β	0.5923
Stirring speed → particle size, β	−0.3771
Chitosan → encapsulation efficiency, β	0.7048
Alginate → encapsulation efficiency, β	0.4995



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Figure 1. Spearman Correlation Matrix of Formulation Response Variables

The correlation matrix revealed strong positive associations of desirability with drug loading (ρ = 0.86) and encapsulation efficiency (ρ = 0.51). Drug retention showed predominantly negative correlations with other formulation responses, indicating distinct stability-related behavior

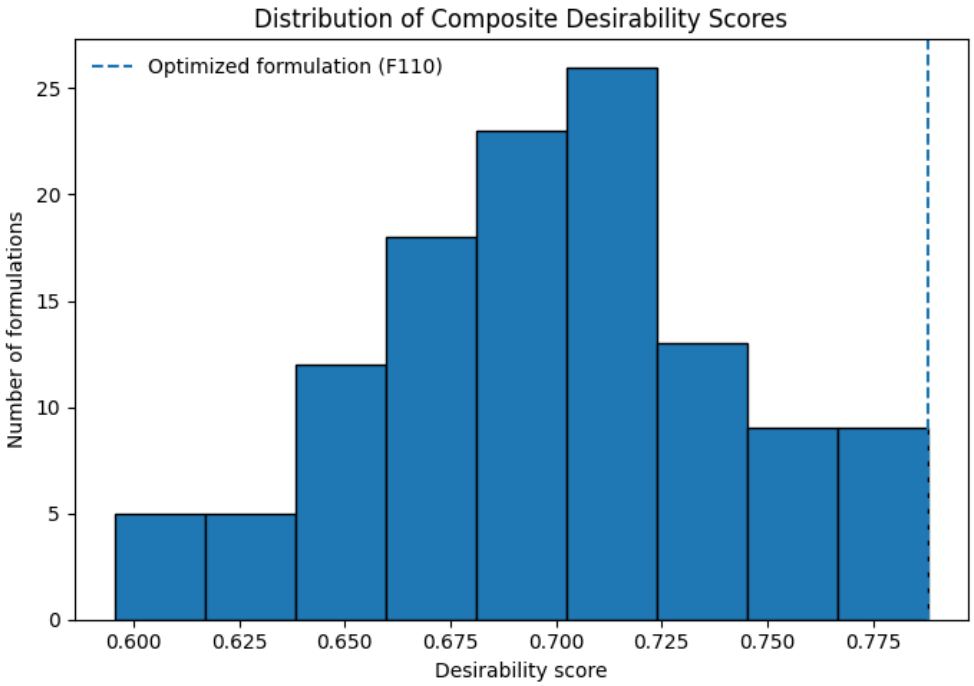


Figure 2. Distribution of Composite Desirability Scores Across Biopolymer Formulations

Composite desirability scores varied across the 120 formulations, with most concentrated around 0.67–0.73. Formulation F110 achieved the maximum desirability score of 0.7879, confirming its selection as the optimized formulation for subsequent evaluation

3.2 Physicochemical Characteristics of the Optimized Formulation

The optimized F110 formulation showed desirable physicochemical properties as presented in Table 2. The particle size was 209.0 nm with 0.252 PDI and the zeta potential was 23.9 mV. The highest drug-loading value (19.1%) for all the investigated formulations was achieved, while the encapsulation efficiency was 86.5%. The production yields were also highest at 96.0%.

The optimized formulation additionally exhibited a swelling index of 208.6% and simulated 90-day drug retention of 93.3%. F110 showed the highest loading amount (100th percentile) and the highest desirability score, and the highest encapsulation efficiency in the formulation set.

Table 2. Physicochemical profile of optimized formulation F110

Physicochemical parameter	**Value**
Particle size (nm)	209.0
PDI	0.252
Zeta potential (mV)	23.9
Encapsulation efficiency (%)	86.5
Drug loading (%)	19.1
Production yield (%)	96.0
Swelling index (%)	208.6
Drug retention at 90 days (%)	93.3
Drug-loading percentile	100th
Desirability percentile	100th

3.3 pH-Dependent Drug Release and Release Kinetics

Significantly different drug release characteristics were shown between free doxorubicin and optimized drug nanoparticles. Free drug has a rapid release, as noted in Table 3, 45.33% of which was released in 0.5 h and the T50 was 0.650 h. Nanoparticle incorporation, on the other hand, resulted in T50 of 3.301 h at pH 5.5 and 6.632 h at pH 7.4, demonstrating a significant sustained release effect.

The optimized formulation was also shown to have a pH-dependent release. At 4 h, cumulative release was 54.90% at pH 5.5 compared with 40.37% at pH 7.4. The highest difference was found for the 8-hour release at pH 5.5 (72.13%) and pH 7.4 (55.13%). Figure 3 shows then that the normalized AUC would be higher under acidic conditions.

The first-order reaction model was the most appropriate model for the entire release curves. The release exponent of the Korsmeyer–Peppas model was 0.4953 at pH 5.5 and 0.4835 at pH 7.4 during the initial phase of the release of nanoparticles, which indicated anomalous/non-Fickian transport.

Table 3. Drug-release characteristics and kinetic-model parameters

Release parameter	Free drug, pH 7.4	Optimized NP, pH 5.5	Optimized NP, pH 7.4
Release at 0.5 h (%)	45.33	18.60	14.73
Release at 4 h (%)	93.00	54.90	40.37
Release at 8 h (%)	97.90	72.13	55.13
Release at 24 h (%)	99.40	93.77	78.30
Release at 72 h (%)	98.27	98.47	96.73
Normalized AUC (%)	97.132	88.572	78.844
T50 (h)	0.650	3.301	6.632
Best full-profile model	First-order	First-order	First-order
R^2	0.9895	0.9728	0.9639
RMSE	3.1297	5.5475	6.0999
AIC	29.1007	41.6936	43.7819
Korsmeyer–Peppas n	—	0.4953	0.4835
Initial release mechanism	—	Anomalous/non-Fickian	Anomalous/non-Fickian

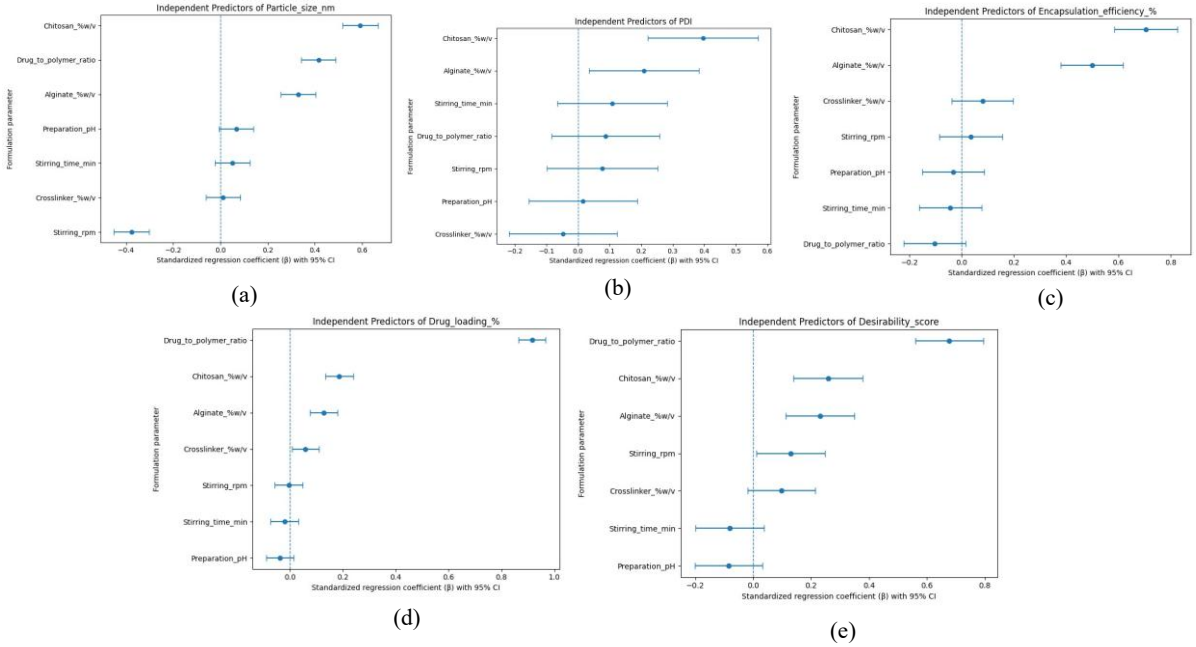


Figure 3. Multivariable Analysis of Independent Formulation Predictors of Critical Nanoparticle Characteristics

Forest plots show standardized regression coefficients with 95% confidence intervals for predictors of (a) particle size, (b) PDI, (c) encapsulation efficiency, (d) drug loading, and (e) composite desirability, highlighting the principal formulation determinants

3.4 Cellular Uptake and Cytotoxic Response

A cellular efficacy analysis showed treatment- and dose-dependency. Nanoparticles loaded with doxorubicin generally exhibited reduced cell viability as compared to free doxorubicin at a number of therapeutic relevant concentrations as summarized in Table 4. For the loaded nanoparticles, viability was reduced to 43.30% at 24 h and 2.5 µg/mL, when compared to 56.78% for free drug. The following figure 4 also indicates that, at 48 h, there were significant reductions for 1.0, 2.5 and 5.0 µg/mL concentrations.

Nanoparticle-mediated delivery always produced higher uptake into cells. For instance, when the concentration of loaded nanoparticles was 10 µg/mL and the time was 24 h, the cellular uptake was 79.73 ng/10^6 cells while the cellular uptake for free doxorubicin was 51.60 ng/10^6 cells. This improvement was sustained at 48 h. The results show that there was a higher availability of intracellular drug with higher loading of nanoparticles and that the higher cytotoxic response was observed at certain concentrations.

Table 4. Representative cell viability and cellular uptake comparisons

Exposure	Dose (µg/mL)	Endpoint	Loaded NP	Free doxorubicin	FDR-adjusted significance
24 h	2.5	Cell viability (%)	43.30	56.78	Significant
48 h	1.0	Cell viability (%)	62.60	71.75	Significant
48 h	2.5	Cell viability (%)	35.93	44.05	Significant
48 h	5.0	Cell viability (%)	18.18	25.95	Significant
24 h	0.1	Cellular uptake (ng/10^6 cells)	38.15	25.63	Significant
24 h	2.5	Cellular uptake (ng/10^6 cells)	63.53	38.63	Significant
24 h	10.0	Cellular uptake (ng/10^6 cells)	79.73	51.60	Significant
48 h	10.0	Cellular uptake (ng/10^6 cells)	79.90	55.73	Significant

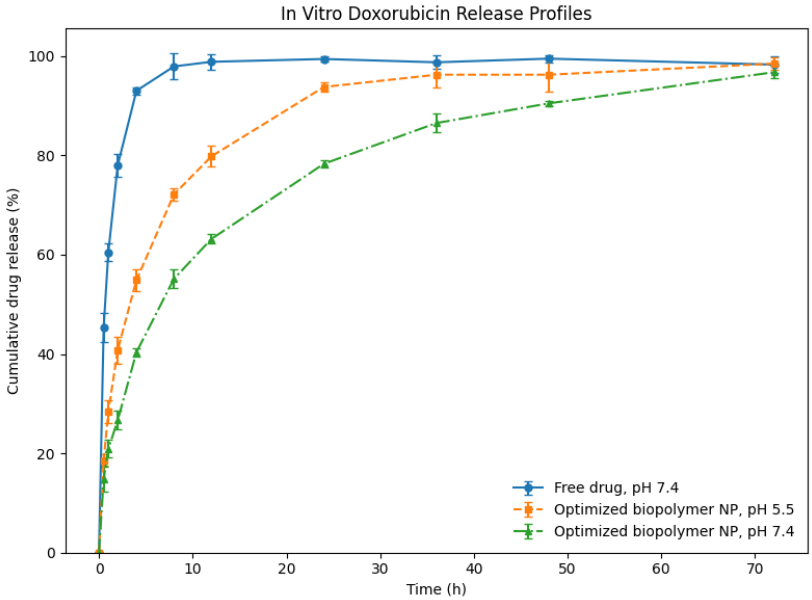


Figure 4. In Vitro Doxorubicin Release Profiles of Free Drug and Optimized Biopolymer Nanoparticles

Free doxorubicin exhibited rapid release, whereas optimized nanoparticles produced sustained, pH-dependent release over 72 h. Drug release was faster at pH 5.5 than at pH 7.4, demonstrating controlled and environmentally responsive release behavior

3.5 Biochemical Evaluation of Therapeutic Response

Biochemical endpoints responded well to treatment and dose—for more detail see Table 5. The ROS responses were significantly higher when the nanoparticles were loaded than the free doxorubicin under most of the dose and exposure conditions. The loaded formulation resulted in an increase in ROS at 48 h and 5.0 µg/mL to 168.28% of control while free doxorubicin was 141.30% of control.

The same trend was observed in the activity of Caspase-3. The activity of caspase-3 was enhanced by 2.47-fold with loaded nanoparticles at 24 h and 5.0 µg/mL, while the free drug was enhanced at 2.02-fold. At 48 h and 10 µg/mL, the corresponding values were 2.93- and 2.43-fold. The results of the factorial analysis showed that after FDR correction there was a significant treatment-, dose-, and treatment × dose-effect for biochemical responses to the factors ROS and caspase-3, as demonstrated in figure 5.

Table 5. Representative biochemical responses to loaded and free doxorubicin

Exposure	Dose (µg/mL)	Endpoint	Loaded NP	Free drug	FDR-adjusted significance
24 h	0.1	ROS (% of control)	115.05	101.28	Significant
24 h	2.5	ROS (% of control)	148.38	130.25	Significant
24 h	10.0	ROS (% of control)	169.60	150.08	Significant
48 h	5.0	ROS (% of control)	168.28	141.30	Significant
24 h	0.5	Caspase-3-fold change	1.65	1.24	Significant
24 h	5.0	Caspase-3-fold change	2.47	2.02	Significant
48 h	2.5	Caspase-3-fold change	2.22	1.83	Significant
48 h	10.0	Caspase-3-fold change	2.93	2.43	Significant

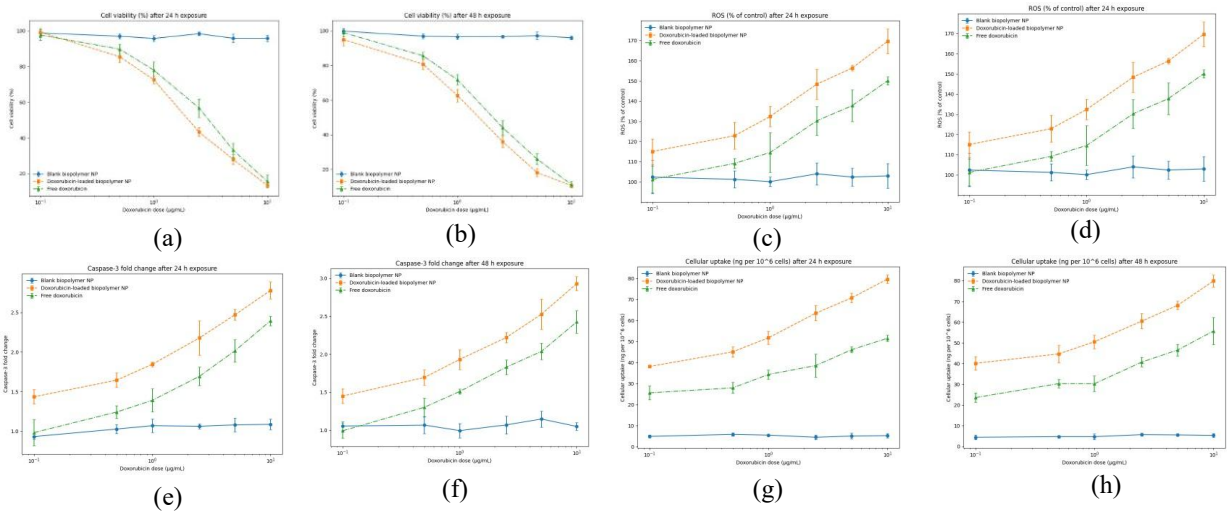


Figure 5. Dose- and Time-Dependent Biochemical and Cellular Responses to Doxorubicin-Loaded Biopolymer Nanoparticles

Dose-response profiles show reduced cell viability (a–b), increased ROS generation (c–d), enhanced caspase-3 activity (e–f), and greater cellular uptake (g–h). Loaded nanoparticles generally produced stronger responses than free doxorubicin across 24- and 48-hour exposures.

3.6 Stability of the Optimized Formulation

The optimized formulation was more physically and chemically stable at 4 °C than at 25 ° C. As shown in Table 6, after 90 days particle size increased to 215.00 ± 2.14 nm at 4 °C but to 220.33 ± 1.29 nm at 25 °C. PDI increased to 0.273 ± 0.003 under refrigerated storage and to 0.311 ± 0.010 at room temperature.

Drug retention was also found to be higher under refrigerated condition (98.20 ± 0.95%) than under normal condition (94.73 ± 1.12%). The day-90 comparisons were all statistically significant following Holm adjustment.

The particle size increased by 2.95% at 4 °C and 5.15% at 25 °C compared to the baseline, while the PDI increased by 8.61% and 24.40%, respectively. Encapsulation and drug retention efficiencies were reduced by 1.03% and 2.64% at 4 °C, respectively, and 4.69% and 5.24% at 25 °C, respectively. It was found that refrigerated storage is the more desirable option for retaining the properties of nanoparticles.

Table 6. Ninety-day stability of the optimized formulation

Stability endpoint	4 °C, mean ± SD	25 °C, mean ± SD	Change at 4 °C (%)	Change at 25 °C (%)	Holm-adjusted result
Particle size (nm)	215.00 ± 2.14	220.33 ± 1.29	+2.95	+5.15	Significant
PDI	0.273 ± 0.003	0.311 ± 0.010	+8.61	+24.40	Significant
Encapsulation efficiency (%)	86.57 ± 0.65	82.57 ± 0.60	−1.03	−4.69	Significant
Drug retention (%)	98.20 ± 0.95	94.73 ± 1.12	−2.64	−5.24	Significant

4. DISCUSSION

The present simulation-based study showed that a chitosan–alginate biopolymer nanoparticle system has the potential to enhance the physicochemical and therapeutic characteristics of the doxorubicin. F110 showed the maximum composite desirability value of 0.7879 among 120 potential formulations, encapsulating 86.5% of the drug, loading 19.1% of it, and yielding 96.0% of the product. The size, loading of nanoparticles, surface characteristics, and therefore the performance of drug delivery of the nanoparticles are important factors and are directly determined by the composition of the polymer and its fabrication methods (Eltaib, 2025). In line with this, chitosan and alginate were important independent factors for a number of critical formulation factors. The significance of alginate concentration, crosslinking, and fabrication parameters in pharmaceutical systems where it is used for controlling the characteristics and the encapsulation has also been highlighted (Lai et al., 2024).

The optimized formulation showed the particle size of 209 nm, PDI of 0.252, and Zeta Potential of 23.9 mV along with an optimized drug incorporation. These properties are relevant since they affect the size, surface properties, and organization of the matrix, which have significant impacts on colloidal behavior, cellular interaction, and drug release from the nanoparticles (Petrovic et al., 2024). The drug-to-polymer ratio had the highest value for drug loading (0.9159) and composite desirability (0.6776) among all the parameters studied. These results justify the rational control of the proportion of drugs in the formulation to develop smart nanocarriers, which heavily rely on the interactions between the therapeutic payload and the architecture of the nanocarriers (Lombardo et al., 2019).

A key discovery was the sustained and pH dependent drug release. The free doxorubicin showed fast release with T50 = 0.650 h, while the incorporation in nanoparticles increased the T50 to 3.301 h at pH 5.5 and 6.632 h at pH 7.4. The complete release profiles fitted well to first-order kinetics. Mathematical kinetic models are valuable tools for elucidating release patterns and assessing the roles of diffusion, matrix relaxation, and other processes (Lakshani et al., 2023). The Korsmeyer–Peppas exponents of 0.4953 and 0.4835, respectively, indicated anomalous/non-Fickian behavior during early release, implying that combined diffusion and polymer relaxation occurred. The therapeutic release from polymeric matrices can be significantly modulated by polymer hydration, degradation and structural changes (Visan et al., 2021).

Release was always faster under the acidic conditions; the normalized AUC was 88.572% at pH 5.5 and 78.844% at pH 7.4. The behavior of formulations may be influenced by environmental pH and consequently by the influence on polymer ionization and matrix interactions (Lukić et al., 2021). Such a discovery is of particular interest to stimuli-responsive delivery strategies, which utilize the physicochemical changes of the environment to control therapeutic release (Li et al., 2019).

The biochemical results also showed increased simulated therapeutic activity. The doxorubicin-loaded nanoparticles in general elevated the uptake, ROS generation, and caspase-3 activity in cells at selected doses and had a greater impact on cell viability. One of the most significant characteristics of advanced nanocarriers is the ability to facilitate the delivery of drugs into cells, which can increase and improve the therapeutic effects at the cellular level (Spicer et al., 2018). Regulation of intracellular oxidative and metabolic pathways is also being increasingly explored as a strategy to enhance therapeutic response (Liu et al., 2025). These associated endpoints, however, do not individually provide an apoptotic mechanism.

Finally, refrigerated storage ensured good formulation integrity. Environmental conditions and degradation processes can have a significant impact on the stability of the polymer and its interactions with the biopolymer can affect long-term functionality in the formulation (Visan et al., 2021; Nwankwo et al., 2025). Collectively, the results support the potential of the optimized biopolymer system for controlled drug delivery. However, all the observations were obtained from simulated data and experimental physicochemical, cellular and in vivo validation is necessary for therapeutic effectiveness to be proven.

5. CONCLUSION

The study was successful in showing that a controlled drug delivery system using a chitosan–alginate biopolymer-based nanoparticles have the potential to offer improved therapeutic performance. F110 was selected as the best formulation according to the multi-response optimization, which showed a desirable balance of the particle properties, EE, drug loading, production yield and composite desirability. The critical quality attributes were greatly affected by the formulation composition, including drug to polymer ratio and chitosan and alginate concentrations. Optimized nanoparticles showed sustained and pH-responsive release of doxorubicin, and this release was prolonged and accelerated in acidic conditions, respectively, compared to the free drug. Further, release kinetics suggest first-order behaviour in all profiles and anomalous/non-Fickian transport in the initial phase of release of nanoparticles. Biochemical assessment showed greater cellular uptake with higher generation of reactive oxygen species and activity of caspase-3 at optimized concentrations and greater decrease in cell viability suggesting better intracellular therapeutic efficacy. Moreover, particle size, drug retention, polydispersity and encapsulation efficiency were maintained better after refrigerated storage for 90 days than in the other storage methods. Together these results provide a consistent formulation–release–response relationship for the imagined delivery platform. The study, however, experimental characterization, mechanistic validation, pharmacokinetic evaluation, safety studies and in vivo efficacy studies must be conducted to validate the therapeutic and translational potential of the study.

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