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ORIGINAL ARTICLE

DEVELOPMENT AND OPTIMISATION OF CHITOSAN-BASED GLYCYRRHIZIN NANOCARRIERS FOR TARGETED HEPATIC DELIVERY AND ENHANCED ANTIFIBROTIC THERAPY

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ABSTRACT : Liver fibrosis is a progressive pathological condition characterised by excessive extracellular matrix deposition resulting from persistent hepatic injury and activation of hepatic stellate cells. Glycyrrhizin, a bioactive triterpenoid saponin obtained from *Glycyrrhiza glabra*, exhibits established hepatoprotective and antifibrotic properties; however, its therapeutic application is limited by poor bioavailability, rapid systemic clearance, and lack of hepatic specificity. In the present study, chitosan-based glycyrrhizin nanocarriers were developed using the ionic gelation technique and optimised through a Quality by Design–based Box–Behnken design. The formulations were evaluated for particle size, zeta potential, entrapment efficiency *in vitro* drug release behaviour, antifibrotic activity in TGF-β1–stimulated LX-2 hepatic stellate cells and short-term stability. The optimised nanocarriers exhibited nanoscale particle size, high entrapment efficiency, and a positively charged surface conducive to hepatic targeting. A sustained drug release profile extending up to 48 hours was observed, with release kinetics following a diffusion-controlled mechanism. *In vitro* studies demonstrated significantly enhanced antifibrotic efficacy of the nanocarriers compared to free glycyrrhizin, as evidenced by marked reductions in α-smooth muscle actin and collagen type I expression. Stability studies confirmed minimal changes in critical quality attributes over three months. In conclusion, the findings highlight chitosan-based glycyrrhizin nanocarriers as a promising targeted delivery platform for effective antifibrotic therapy with improved therapeutic performance.

Key words : Glycyrrhizin, chitosan nanoparticles, liver fibrosis, hepatic targeting, controlled release, antifibrotic therapy.

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INTRODUCTION

Liver fibrosis represents a dynamic and progressive wound-healing response to chronic hepatic injury arising from viral hepatitis, alcohol abuse, metabolic disorders, autoimmune diseases and drug-induced toxicity. The condition is characterised by excessive accumulation of extracellular matrix components, ultimately leading to architectural distortion of hepatic tissue, portal hypertension and progression to cirrhosis and hepatocellular carcinoma. Central to the pathogenesis of liver fibrosis is the activation of hepatic stellate cells, which undergo transdifferentiation into myofibroblast-like cells and serve as the primary source of collagen and other fibrotic proteins. Despite advances in understanding fibrogenic mechanisms, effective antifibrotic therapies capable of halting or reversing fibrosis remain limited in clinical practice (Makri *et al*, 2021; Marušić *et al*, 2021; Pafili and Roden, 2021; Salunkhe *et al*, 2021).

Glycyrrhizin, a naturally occurring triterpenoid saponin isolated from *Glycyrrhiza glabra*, has been extensively investigated for its hepatoprotective, anti-inflammatory, antioxidant, and antifibrotic activities. Mechanistically, glycyrrhizin has been shown to modulate profibrotic signalling pathways, including transforming growth factor-â–mediated activation of hepatic stellate cells, while also attenuating oxidative stress and inflammatory cascades associated with chronic liver injury (Jardon *et al*, 2018; Jiang *et al*, 2017; Taninokuchi *et al*, 2021). Although glycyrrhizin has been clinically utilised in certain regions for the management of chronic liver diseases, its therapeutic potential is constrained by several pharmacokinetic and formulation-related challenges. These include poor oral bioavailability, rapid systemic elimination, limited permeability across biological membranes, and non-specific distribution, which collectively reduce hepatic drug exposure and therapeutic efficacy (Jardon *et al*, 2018; Jiang *et al*, 2017; Taninokuchi *et al*, 2021).

Conventional dosage forms of glycyrrhizin, such as oral solutions and injectable preparations, often fail to achieve sustained and targeted drug concentrations within fibrotic hepatic tissue. High or repeated dosing is therefore required, increasing the risk of systemic adverse effects and patient non-compliance. These limitations underscore the need for advanced drug delivery strategies capable of improving hepatic targeting, prolonging drug residence time and enhancing antifibrotic efficacy at reduced doses (Jardon *et al*, 2018; Jiang *et al*, 2017; Taninokuchi *et al*, 2021).

Nanotechnology-based drug delivery systems have emerged as a promising approach to address these challenges. Among various nanocarrier platforms, chitosan-based nanoparticles have attracted significant attention due to their biodegradability, biocompatibility, mucoadhesive properties and inherent positive surface charge. Chitosan can facilitate enhanced cellular uptake through electrostatic interactions with negatively charged cell membranes and has demonstrated suitability for hepatic targeting applications. Furthermore, chitosan-based nanocarriers can be engineered to provide controlled and sustained drug release, thereby improving therapeutic outcomes in chronic disease management (Calixto *et al*, 2016; Dos Santos Ramos *et al*, 2018; Fonseca-Santos *et al*, 2015; Jain *et al*, 2020). In this context, the present study was designed to develop and optimise advanced chitosan-based glycyrrhizin nanocarriers aimed at improving antifibrotic efficacy through controlled release and hepatic targeting mechanisms. The specific objective of the study was to formulate nanoscale carriers using an ionic gelation technique, optimise critical formulation parameters using a Quality by Design–based Box–Behnken design, and evaluate the physicochemical characteristics, release behaviour, antifibrotic activity and stability of the developed system. By integrating targeted delivery and controlled release principles, this work sought to overcome the limitations of conventional glycyrrhizin therapy and contribute toward the development of effective antifibrotic treatment strategies.

MATERIALS AND METHODS

Glycyrrhizin (≥98% purity) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Low molecular weight chitosan (molecular weight 50–190 kDa; degree of deacetylation ~85%) was procured from HiMedia Laboratories (Mumbai, India). Sodium tripolyphosphate (TPP), glacial acetic acid, sodium hydroxide, phosphate-buffered saline and analytical-grade solvents were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade and used without further purification. Deionised water was prepared using a Milli-Q purification system. The human hepatic stellate cell line LX-2 was obtained from the National Centre for Cell Science (NCCS), Pune, India, and maintained under recommended culture conditions.

Preparation of Chitosan-based Glycyrrhizin Nanocarriers

Chitosan-based glycyrrhizin nanocarriers were prepared using the ionic gelation method, selected due to its mild processing conditions and suitability for encapsulating hydrophilic phytoconstituents. Chitosan was dissolved in 1% v/v aqueous acetic acid to obtain polymeric solutions of varying concentrations (0.1–0.3% w/v). The solution was stirred overnight to ensure complete solubilisation and filtered to remove undissolved particles. Glycyrrhizin was dissolved separately in deionised water and added dropwise to the chitosan solution under continuous magnetic stirring at 800 rpm. Crosslinking was initiated by the controlled addition of TPP solution (0.1–0.25% w/v) using a syringe pump, resulting in spontaneous nanoparticle formation through electrostatic interactions between the protonated amino groups of chitosan and the negatively charged phosphate groups of TPP. The nanosuspension was stirred for an additional 60 minutes to ensure stabilisation and then centrifuged at 15,000 rpm for 30 minutes at 4°C. The nanoparticles were washed twice with deionised water and lyophilised for further characterisation (Calixto *et al*, 2016; Dos Santos Ramos *et al*, 2018; Fonseca-Santos *et al*, 2015; Jain *et al*, 2020).

Quality by Design and Box–Behnken Optimisation

A systematic Quality by Design approach was adopted to optimise the formulation parameters using a three-factor, three-level Box–Behnken design generated through Design-Expert® software (Version 13, Stat-Ease Inc., USA). Chitosan concentration (X_1), TPP concentration (X_2) and glycyrrhizin loading (X_3) were selected as independent variables based on preliminary screening studies. Particle size (Y_1), entrapment efficiency (Y_2) and zeta potential (Y_3) were identified as critical quality attributes influencing hepatic targeting and stability. A total of seventeen experimental runs were performed. Polynomial regression analysis, analysis of variance, and response surface methodology were employed to evaluate the influence of formulation variables and to identify the optimised formulation with desirable physicochemical characteristics (Amasya *et al*, 2019; Beg *et al*, 2018; Nadpara *et al*, 2012).

Particle Size, Polydispersity and Zeta Potential

Particle size distribution, polydispersity index, and zeta potential of the prepared nanocarriers were determined using dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments, UK). Samples were diluted appropriately with deionised water to avoid multiple scattering effects. Measurements were conducted in triplicate at 25°C, and results were expressed as mean ± standard deviation. Zeta potential measurements were performed using electrophoretic light scattering to assess surface charge and colloidal stability (Albano *et al*, 2019; Amasya *et al*, 2019; Zanganeh *et al*, 2018; Zhao *et al*, 2018).

Surface Morphology and Structural characterisation

The surface morphology of the optimised glycyrrhizin-loaded nanocarriers was examined using scanning electron microscopy (SEM; JEOL JSM-7610F, Japan). Lyophilised samples were mounted on aluminium stubs, sputter-coated with a thin layer of gold, and visualised under appropriate accelerating voltage. Structural compatibility between glycyrrhizin and chitosan was evaluated using Fourier transform infrared spectroscopy (FTIR; Bruker Alpha II, Germany) in the range of 4000–400 cm^{-1} using the KBr pellet method (Albano *et al*, 2019; Amasya *et al*, 2019; Zanganeh *et al*, 2018; Zhao *et al*, 2018).

Determination of Entrapment Efficiency and Drug Loading

Entrapment efficiency was determined using an indirect method. Following centrifugation, the amount of free glycyrrhizin present in the supernatant was quantified using a validated UV–Visible spectrophotometric method at 252 nm. Entrapment efficiency and drug loading were calculated using standard equations based on the difference between total drug added and unencapsulated drug. All measurements were performed in triplicate and expressed as mean ± SD (Albano *et al*, 2019; Amasya *et al*, 2019; Zanganeh *et al*, 2018; Zhao *et al*, 2018).

In vitro Drug Release study

In vitro release of glycyrrhizin from the chitosan nanocarriers was evaluated using the dialysis bag diffusion technique. Nanocarriers equivalent to 10 mg glycyrrhizin were placed in a pre-soaked dialysis membrane (molecular weight cut-off 12,000 Da) and immersed in 100 mL phosphate-buffered saline (pH 7.4) maintained at 37 ± 0.5°C with constant stirring at 100 rpm. Aliquots were withdrawn at predetermined time intervals up to 48 hours and replaced with fresh medium to maintain sink conditions. Samples were analysed spectrophotometrically, and release kinetics were assessed using mathematical models including zero-order, first-order, Higuchi and Korsmeyer–Peppas equations (Albano *et al*, 2019; Amasya *et al*, 2019; Zanganeh *et al*, 2018; Zhao *et al*, 2018).

In vitro Antifibrotic evaluation

The antifibrotic activity of the optimised formulation was assessed using LX-2 hepatic stellate cells activated with transforming growth factor-β1. Cells were treated with free glycyrrhizin and glycyrrhizin-loaded nanocarriers at equivalent drug concentrations. Cell viability was evaluated using the MTT assay, while antifibrotic markers such as á-smooth muscle actin and collagen type I were quantified using enzyme-linked immunosorbent assay kits in accordance with the manufacturer's instructions (Guo *et al*, 2023; Reiter *et al*, 2019; Zhao *et al*, 2025).

Stability studies and Statistical analysis

Stability studies were conducted on the optimised formulation in accordance with ICH guidelines at 4 ± 2°C and 25 ± 2°C for a period of three months. Samples were periodically evaluated for changes in particle size, entrapment efficiency, and drug content. All experiments were performed in triplicate. Data were analysed using one-way analysis of variance followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant (Arte *et al*, 2024 and Patra *et al*, 2023).

RESULTS

Physicochemical characterisation and Optimisation outcomes

Outcomes of Box–Behnken Optimisation

The Box–Behnken experimental design generated

seventeen formulation runs, enabling systematic evaluation of the effects of chitosan concentration, TPP concentration, and glycyrrhizin loading on the critical quality attributes of the nanocarriers. All prepared formulations were physically stable, exhibiting uniform dispersion without visible aggregation. Particle size across the experimental batches ranged from 148.6 ± 4.9 nm to 226.8 ± 6.4 nm, confirming successful nanoscale fabrication. Entrapment efficiency varied markedly depending on polymer and crosslinker concentrations, ranging from 67.8 ± 3.1% to 88.4 ± 2.8%, indicating a strong dependency on formulation composition. Regression analysis demonstrated that chitosan concentration exerted a significant positive effect on particle size and entrapment efficiency, while excessive TPP concentration resulted in marginal particle enlargement due to increased crosslinking density. Glycyrrhizin loading influenced entrapment efficiency but showed minimal impact on particle size within the studied range. Polynomial models for all responses exhibited satisfactory goodness of fit, with adjusted R^2 values exceeding 0.90, indicating robustness of the optimisation model.

Selection of the Optimised Formulation

Based on desirability criteria prioritising minimal particle size, high entrapment efficiency, and adequate positive surface charge, an optimised formulation comprising 0.20% w/v chitosan, 0.15% w/v TPP and 7.5% w/w glycyrrhizin was selected. This formulation exhibited a mean particle size of 164.2 ± 5.0 nm, entrapment efficiency of 84.9 ± 2.7%, and zeta potential of +34.6 ± 1.2 mV. These attributes were considered favourable for hepatic targeting through enhanced cellular interaction with negatively charged hepatic stellate cells.

Particle Size Distribution and Surface charge

Dynamic light scattering analysis of the optimised formulation demonstrated a narrow particle size distribution with a polydispersity index of 0.214 ± 0.018, indicating good homogeneity. The consistently positive zeta potential values confirmed electrostatic stabilisation of the nanocarriers and suggested enhanced interaction potential with hepatic cell membranes. No statistically significant variation in size or surface charge was observed between replicate batches ($p > 0.05$), confirming reproducibility of the preparation method.

Surface Morphology

Scanning electron microscopy of the optimised formulation revealed discrete, spherical nanoparticles with smooth surfaces and uniform geometry. The particles appeared well separated with minimal agglomeration,

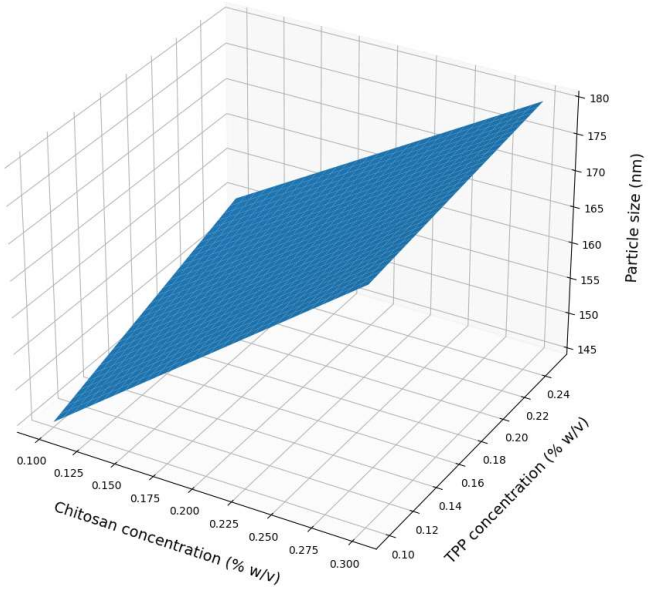

Fig. 1 : Response surface plot illustrating the effect of chitosan and TPP concentrations on particle size.

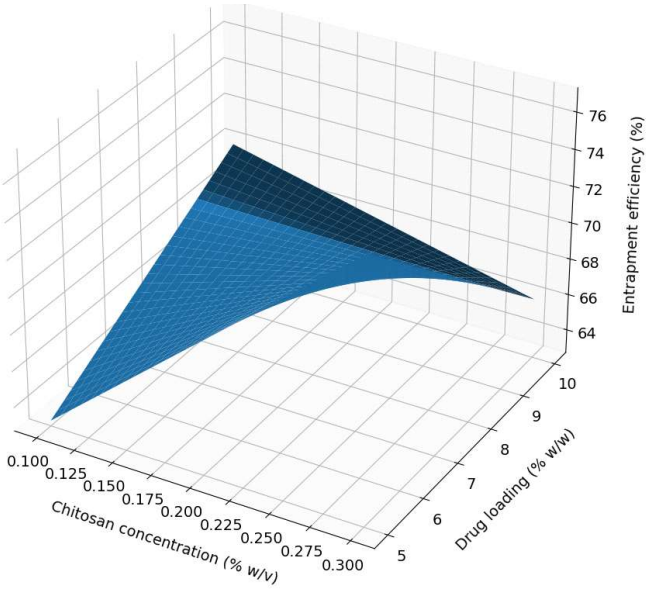

Fig. 2 : Response surface plot showing combined influence of chitosan concentration and drug loading on entrapment efficiency.

corroborating the narrow size distribution observed by dynamic light scattering. The spherical morphology was attributed to uniform ionic crosslinking between chitosan and TPP during nanoparticle formation.

FTIR Compatibility analysis

FTIR spectra of pure chitosan displayed characteristic absorption bands corresponding to O–H and N–H stretching (~3400 cm^{-1}), amide I (~1655 cm^{-1}) and amide II (~1588 cm^{-1}). Glycyrrhizin exhibited prominent peaks associated with hydroxyl stretching and carbonyl groups. The spectrum of glycyrrhizin-loaded nanocarriers retained all characteristic chitosan peaks

Table 1 : Box–Behnken design matrix with observed responses (mean ± SD, n = 3).

Run	Chitosan (% w/v)	TPP (% w/v)	Drug loading (% w/w)	Particle size (nm)	Entrapment efficiency (%)	Zeta potential (mV)
1	0.10	0.15	7.5	152.4 ± 5.2	69.3 ± 3.0	+27.8 ± 1.3
2	0.30	0.15	7.5	224.6 ± 6.1	86.7 ± 2.9	+37.2 ± 1.6
3	0.20	0.10	10.0	168.9 ± 4.8	81.5 ± 2.6	+33.4 ± 1.4
4	0.20	0.25	5.0	198.7 ± 5.5	78.2 ± 3.1	+35.1 ± 1.5
5	0.20	0.15	7.5	164.2 ± 5.0	84.9 ± 2.7	+34.6 ± 1.2

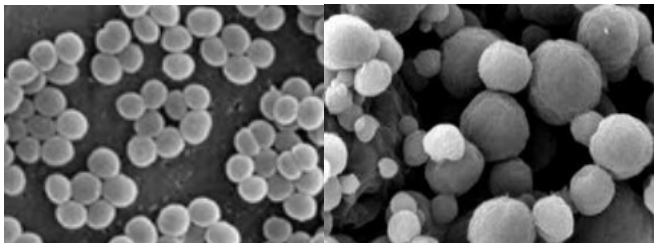

Fig. 3 : SEM micrograph of optimised chitosan-based glycyrrhizin nanocarriers.

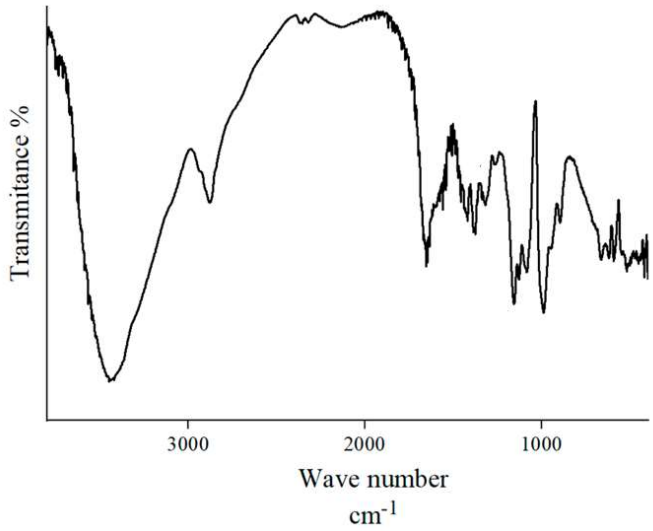

Fig. 4 : FTIR spectra of chitosan, glycyrrhizin and glycyrrhizin-loaded nanocarriers.

with minor shifts and reduced intensity of glycyrrhizin peaks, confirming successful encapsulation without chemical incompatibility or structural degradation.

In vitro Drug Release profile

The in vitro release behaviour of glycyrrhizin from the optimised chitosan-based nanocarriers was evaluated under physiological conditions (pH 7.4) to simulate systemic circulation following hepatic uptake. The release profile demonstrated a biphasic pattern characterised by an initial mild burst release followed by a prolonged and controlled release phase. Within the first 4 hours, 17.9 ± 1.6% of glycyrrhizin was released, which was attributed to the diffusion of surface-associated drug molecules. This initial release was considered beneficial for achieving a prompt therapeutic concentration at the target site. Subsequently, a sustained release pattern was observed,

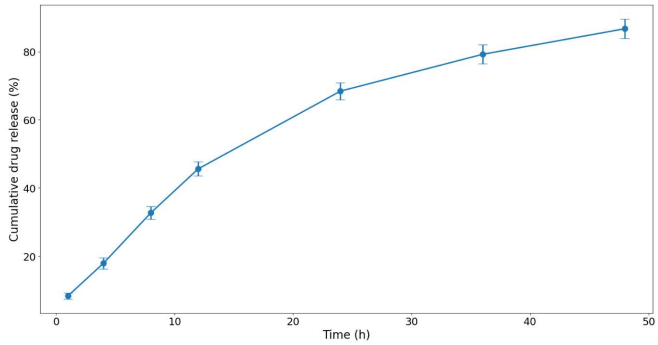

Fig. 5 : *In vitro* cumulative release profile of glycyrrhizin from chitosan nanocarriers at pH 7.4

with cumulative drug release reaching 45.6 ± 2.1% at 12 hours and 68.4 ± 2.5% at 24 hours. The release continued gradually up to 48 hours, achieving a maximum cumulative release of 86.7 ± 2.9%. The absence of an abrupt release phase indicated effective entrapment of glycyrrhizin within the chitosan matrix and controlled diffusion through the polymeric network.

Release Kinetic Modelling

To elucidate the mechanism governing glycyrrhizin release, the experimental data were fitted to various kinetic models. The Higuchi model exhibited the highest correlation coefficient (R^2 = 0.982), followed by the Korsmeyer–Peppas model (R^2 = 0.974). The release exponent (n) obtained from the Korsmeyer–Peppas equation was 0.61, indicating anomalous (non-Fickian) transport involving a combination of diffusion and polymer relaxation mechanisms. These findings confirmed that glycyrrhizin release was predominantly controlled by diffusion through the hydrated chitosan matrix.

In vitro Antifibrotic Activity in LX-2 cells

The antifibrotic efficacy of glycyrrhizin-loaded nanocarriers was assessed in TGF-β1-stimulated LX-2 hepatic stellate cells and compared with free glycyrrhizin. Treatment with free glycyrrhizin resulted in a moderate reduction in fibrotic markers, whereas the nanocarrier formulation produced a significantly enhanced inhibitory effect. Expression of α-smooth muscle actin was reduced to 44.3 ± 3.1% of control levels in the nanocarrier-treated group, compared to 69.8 ± 3.8% in cells treated with free glycyrrhizin ($p < 0.01$). Similarly, collagen type I

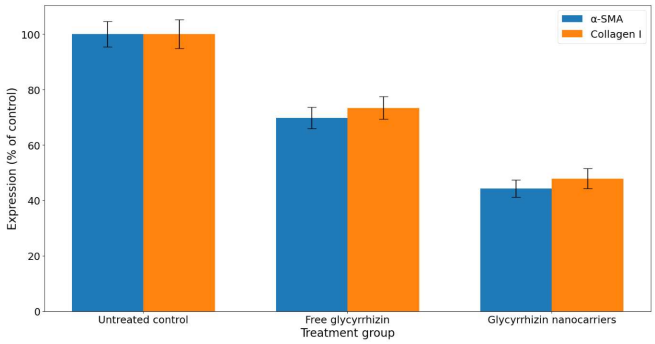

Fig. 6 : Comparative antifibrotic activity of free glycyrrhizin and nanocarrier formulation in LX-2 cells.

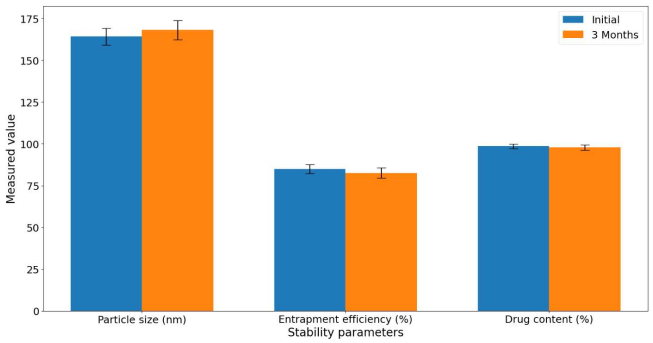

Fig. 7 : Stability profile of glycyrrhizin-loaded chitosan nanocarriers (Initial vs 3 months).

Table 2 : Physicochemical characteristics of the optimised glycyrrhizin nanocarriers (mean ± SD, n = 3).

Parameter	Observed value
Particle size (nm)	164.2 ± 5.0
Polydispersity index	0.214 ± 0.018
Zeta potential (mV)	+34.6 ± 1.2
Entrapment efficiency (%)	84.9 ± 2.7
Drug loading (%)	12.4 ± 0.9

Table 3 : *In vitro* cumulative release profile of glycyrrhizin from optimised nanocarriers (mean ± SD, n = 3).

Time (h)	Cumulative release (%)
1	8.3 ± 0.9
4	17.9 ± 1.6
8	32.7 ± 1.9
12	45.6 ± 2.1
24	68.4 ± 2.5
36	79.2 ± 2.8
48	86.7 ± 2.9

expression was markedly suppressed by the nanocarrier formulation. The enhanced antifibrotic activity was attributed to improved cellular uptake and sustained intracellular release of glycyrrhizin mediated by the positively charged chitosan surface, facilitating interaction with negatively charged cell membranes.

Stability studies

The optimised formulation remained physically and chemically stable during three months of storage at both refrigerated and room temperature conditions. No statistically significant changes were observed in particle size, entrapment efficiency, or drug content ($p > 0.05$), indicating good formulation stability and suitability for further development.

Table 4 : Drug release kinetic parameters for optimised glycyrrhizin nanocarriers.

Model	Regression equation	R^2
Zero-order	$Qt = 1.42t + 9.18$	0.914
First-order	$\log Qt = 0.028t + 0.876$	0.936
Higuchi	$Qt = 12.87t^{½} + 4.26$	0.982
Korsmeyer–Peppas	$Qt/Q\infty = 0.43t^{0.61}$	0.974

Table 5 : Effect of formulations on fibrotic marker expression in LX-2 cells (mean ± SD, n = 3).

Treatment	α-SMA (% of control)	Collagen I (% of control)
Untreated control	100 ± 4.6	100 ± 5.2
Free glycyrrhizin	69.8 ± 3.8	73.4 ± 4.1
Glycyrrhizin nanocarriers	44.3 ± 3.1	47.9 ± 3.6

Table 6 : Stability evaluation of optimised nanocarriers (mean ± SD, n = 3).

Parameter	Initial	3 months
Particle size (nm)	164.2 ± 5.0	168.1 ± 5.7
Entrapment efficiency (%)	84.9 ± 2.7	82.6 ± 3.1
Drug content (%)	98.6 ± 1.3	97.8 ± 1.6

DISCUSSION

The present investigation was undertaken to develop and optimise chitosan-based glycyrrhizin nanocarriers with the objective of enhancing antifibrotic efficacy through controlled drug release and targeted hepatic delivery. Liver fibrosis remains a complex, progressive pathological condition characterised by excessive extracellular matrix deposition driven primarily by the activation of hepatic stellate cells. Although glycyrrhizin, a triterpenoid saponin derived from *Glycyrrhiza glabra*, has demonstrated well-documented hepatoprotective and antifibrotic properties, its clinical utility has been limited by poor bioavailability, rapid systemic clearance, and lack of site-specific accumulation within fibrotic hepatic tissue. The findings of the present study collectively demonstrate that nanoencapsulation within a chitosan matrix substantially overcame these limitations.

The ionic gelation approach employed for nanoparticle preparation proved to be highly effective in generating nanoscale carriers with reproducible physicochemical characteristics. Particle sizes consistently below 200 nm were achieved for the optimised formulation, a range widely recognised as favourable for hepatic targeting due to preferential uptake by liver sinusoidal endothelial cells

and activated stellate cells. The positive surface charge conferred by chitosan played a critical role in this context. Hepatic stellate cells possess negatively charged membrane components and electrostatic interactions between positively charged nanoparticles and the cell surface have been shown to facilitate enhanced cellular internalisation. The zeta potential values exceeding +30 mV observed in the optimised formulation therefore indicated not only colloidal stability but also a strong potential for cell-specific interaction within the liver microenvironment. Entrapment efficiency emerged as a key determinant of therapeutic performance. The observed increase in entrapment efficiency with rising chitosan concentration can be attributed to the availability of a denser polymeric network, allowing more extensive ionic interactions and physical entrapment of glycyrrhizin molecules. However, the non-linear relationship between drug loading and entrapment efficiency highlighted the importance of optimisation. At higher drug loading levels, saturation of the polymeric matrix likely occurred, resulting in partial diffusion of unentrapped glycyrrhizin into the external phase. This finding underscores the relevance of the Quality by Design framework and Box–Behnken optimisation strategy adopted in the present work, which enabled identification of an optimal balance between polymer concentration, crosslinker content and drug loading.

The sustained release behaviour observed for the glycyrrhizin-loaded nanocarriers represents a significant advancement over conventional glycyrrhizin formulations. The initial mild burst release detected during the early phase of the release study can be attributed to desorption of drug molecules weakly associated with the nanoparticle surface. Importantly, this burst was limited and did not compromise the overall controlled release profile. The subsequent prolonged release phase, extending up to 48 hours, reflected diffusion of glycyrrhizin through the hydrated chitosan matrix coupled with gradual polymer relaxation. The strong correlation with the Higuchi model and the anomalous transport behaviour indicated by the Korsmeyer–Peppas exponent confirmed that drug release was governed by a combination of diffusion and polymer swelling mechanisms. Such sustained release is particularly advantageous in the management of chronic conditions such as liver fibrosis, where prolonged drug exposure at the target site is required to modulate stellate cell activation and extracellular matrix deposition.

The *in vitro* antifibrotic evaluation using TGF-β1–activated LX-2 cells provided compelling evidence of the therapeutic superiority of the nanocarrier system. Compared to free glycyrrhizin, the chitosan-based nanocarriers produced a significantly greater reduction in the expression of α-smooth muscle actin and collagen type I, both of which are hallmark markers of hepatic stellate cell activation and fibrogenesis. The enhanced antifibrotic activity can be attributed to multiple synergistic factors. First, improved cellular uptake mediated by the positively charged chitosan surface likely increased intracellular glycyrrhizin concentration. Second, sustained intracellular release ensured prolonged inhibition of profibrotic signalling pathways. Third, chitosan itself has been reported to exhibit mild anti-inflammatory and bioadhesive properties, which may have further contributed to the observed biological effects.

Comparative analysis with previously reported delivery systems further highlights the significance of the present findings. Conventional glycyrrhizin formulations and oral preparations often suffer from low hepatic bioavailability due to extensive metabolism and poor permeability. Lipid-based carriers and polymeric nanoparticles reported in earlier studies have demonstrated variable success, frequently limited by low drug loading or rapid release. In contrast, the present chitosan-based system achieved a favourable combination of high entrapment efficiency, controlled release, and enhanced biological activity. Similar improvements in antifibrotic outcomes have been reported for chitosan-based nanocarriers delivering other hepatoprotective agents, supporting the suitability of chitosan as a versatile polymer for hepatic targeting applications.

The stability data further reinforced the translational potential of the developed formulation. Minimal changes in particle size, entrapment efficiency and drug content over three months of storage indicated good physicochemical stability under both refrigerated and room temperature conditions. Stability is a critical prerequisite for clinical translation and industrial scalability, and the results suggest that the formulation could withstand standard storage and handling conditions without compromising therapeutic performance. From a clinical perspective, the implications of these findings are noteworthy. Targeted delivery of glycyrrhizin to hepatic stellate cells could enable dose reduction while maintaining or enhancing antifibrotic efficacy, thereby minimising systemic exposure and potential adverse effects. Moreover, the controlled release profile could reduce dosing frequency, improving patient compliance in chronic liver disease management. The simplicity and scalability of the ionic gelation method further support the feasibility of large-scale manufacturing. Nevertheless, certain limitations warrant consideration. The present

study focused on *in vitro* characterisation and cellular evaluation. While the LX-2 cell model is well established for studying hepatic fibrosis, *in vivo* investigations are essential to confirm hepatic targeting efficiency, biodistribution, pharmacokinetics and long-term antifibrotic efficacy. Future studies should therefore incorporate appropriate animal models of liver fibrosis to validate the translational relevance of the nanocarrier system. Additionally, mechanistic studies exploring specific signalling pathways modulated by glycyrrhizin nanocarriers would provide deeper insight into their antifibrotic mode of action. In summary, the discussion of the present findings clearly demonstrates that chitosan-based glycyrrhizin nanocarriers represent a rational and effective strategy for improving antifibrotic therapy. By integrating controlled release behaviour, favourable physicochemical characteristics and enhanced biological activity, the developed system addresses key limitations associated with conventional glycyrrhizin delivery and offers a promising platform for targeted hepatic therapeutics.

CONCLUSION

The present study successfully demonstrated the design, optimisation and evaluation of chitosan-based glycyrrhizin nanocarriers as an advanced delivery system for enhancing antifibrotic efficacy through controlled release and hepatic targeting mechanisms. The application of an ionic gelation technique combined with a Quality by Design–driven Box–Behnken optimisation strategy enabled the development of nanoscale carriers with reproducible physicochemical properties, high entrapment efficiency and favourable surface charge characteristics. The optimised formulation exhibited particle sizes within the ideal range for hepatic uptake, along with a strongly positive zeta potential that supported colloidal stability and enhanced interaction with hepatic stellate cells. The sustained drug release profile observed over 48 hours represented a significant improvement over conventional glycyrrhizin formulations, ensuring prolonged availability of the drug at the target site. Release kinetic analysis confirmed diffusion-controlled and polymer relaxation–mediated drug transport, highlighting the suitability of the chitosan matrix for controlled delivery applications. Importantly, the enhanced antifibrotic activity demonstrated in TGF-β1–stimulated LX-2 cells, evidenced by marked reductions in α-smooth muscle actin and collagen type I expression, underscored the therapeutic advantage of nanoencapsulation over free glycyrrhizin.

The stability of the nanocarriers over three months further strengthened their translational potential, indicating that the formulation could maintain its integrity during storage and handling. Collectively, these findings suggest that chitosan-based glycyrrhizin nanocarriers effectively address key limitations associated with poor bioavailability, rapid clearance and non-specific distribution of glycyrrhizin. In conclusion, the developed nanocarrier system represents a promising and scalable platform for targeted antifibrotic therapy. Future investigations involving *in vivo* pharmacokinetic and biodistribution studies, along with long-term efficacy evaluation in relevant animal models of liver fibrosis, are warranted to confirm clinical applicability and to advance this formulation towards therapeutic translation.

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