

Optimization of Chitosan Nanoparticle Synthesis Parameters for Enhanced Gamma Oryzanol Loading

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ABSTRACT

The present study focuses on the formulation, characterization, and evaluation of chitosan nanoparticles loaded with gamma oryzanol (CG nanoparticles) aimed at enhancing its antioxidant and therapeutic efficacy. Nanoparticles were synthesized via a solvent evaporation technique using varying ratios of chitosan and drug. The prepared formulations were assessed for percentage yield, drug entrapment efficiency (EE), particle size, polydispersity index (PDI), and morphological characteristics. The percentage yield ranged between 81.45–98.12%, while EE varied from 68.34±3.15% to 94.15±1.24%. Particle size analysis showed nanometric sizes between 710.02±1.20 nm to 904.25±1.12 nm, with PDI values indicating acceptable uniformity for pulmonary delivery. SEM imaging confirmed spherical, moderately agglomerated nanoparticles, and DSC and FTIR analyses indicated significant drug-polymer interaction and amorphous nature of the encapsulated drug. In vitro drug release studies demonstrated sustained release behavior over 24 hours, with CG5 showing the highest cumulative release. Antioxidant potential evaluated via DPPH, ABTS, and FRAP assays confirmed significant free radical scavenging capacity. These findings suggest that CG nanoparticles could be a promising carrier for gamma oryzanol with enhanced therapeutic potential.

Keywords: chitosan nanoparticles, gamma oryzanol, antioxidant activity, drug entrapment efficiency, DPPH, FTIR, DSC, ionotropic gelation

1. Introduction

Diabetes mellitus is one of the most prevalent and challenging chronic metabolic disorders worldwide. It is characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The condition affects carbohydrate, fat, and protein metabolism and, if left untreated, can lead to severe complications such as retinopathy, nephropathy, neuropathy, cardiovascular diseases, and poor wound healing. According to the International Diabetes Federation (IDF), over 537 million adults were living with diabetes in 2021, and the number is expected to rise to 783 million by 2045. This alarming trend underscores the urgent need for effective, safe, and affordable therapeutic strategies for diabetes management.[1,2]

Although various synthetic oral hypoglycemic agents and insulin therapies are available, they are often associated with several limitations, including side effects, high cost, and poor patient compliance. Consequently, attention has shifted toward exploring alternative approaches, particularly those based on natural products and phytochemicals that offer potential therapeutic benefits with minimal adverse effects.[3]

Gamma oryzanol (γ -oryzanol) is a naturally occurring mixture of ferulic acid esters of phytosterols and triterpene alcohols, predominantly found in rice bran oil. It exhibits a wide range of pharmacological properties, including antioxidant, anti-inflammatory, lipid-lowering, and neuroprotective effects [4-7]. Recent studies have also demonstrated its potential role in improving glucose metabolism and insulin sensitivity, suggesting its utility as a promising therapeutic agent for managing diabetes mellitus.[8] However, the clinical application of gamma oryzanol is significantly limited due to its poor aqueous solubility, low gastrointestinal absorption, and rapid metabolic degradation, which collectively result in low systemic bioavailability. To overcome these challenges, novel drug delivery systems are required that can enhance the solubility, stability, and targeted delivery of gamma oryzanol.[9-15]

Nanotechnology-based delivery systems have emerged as a powerful tool to address the limitations associated with conventional dosage forms. Among them, polymeric nanoparticles have gained considerable attention for the delivery of poorly soluble drugs [16,17]. Chitosan, a natural polysaccharide obtained by the deacetylation of chitin, is extensively used in pharmaceutical applications due to its favorable characteristics, including biocompatibility, biodegradability, mucoadhesiveness, and ability to transiently open tight junctions in epithelial cells, thus facilitating enhanced drug absorption across biological membranes[18]

Chitosan nanoparticles can be easily prepared via the ionic gelation method, which involves the electrostatic interaction between the positively charged amino groups of chitosan and negatively charged crosslinkers like sodium tripolyphosphate (TPP) [19]. This technique is advantageous due to its simplicity, mild preparation conditions, and avoidance of harsh organic solvents or high energy input [20]. In this study, we aim to develop chitosan-based nanoparticles encapsulating gamma oryzanol to improve its solubility, protect it from premature degradation, and achieve controlled release for prolonged therapeutic action. The formulated nanoparticles will be extensively characterized for their physicochemical properties, including particle size, zeta potential, encapsulation efficiency, and drug release behavior.[21-27]

2. Materials and Methods

2.1 Materials

High molecular weight chitosan (MW: 350,000 g/mol, deacetylation degree >75%, and viscosity 800–2000 cps), sodium tripolyphosphate (TPP), and ferric ammonium citrate were procured from Sigma-Aldrich Co. (St. Louis, MO, USA). Gamma Oryzanol was obtained from India Mart. All additional materials and solvents employed in this research were of analytical grade.

2.2 Preparation of Chitosan Nanoparticles Loaded with Gamma Oryzanol (CG Nanoparticles)

CG nanoparticles were prepared using the ionotropic gelation method. To create blank nanoparticles, a TPP aqueous solution (final concentration 1 mg/mL) was added to a CG acetic

acid solution. The CG/TPP ratio was 2:1 (weight/weight), and different stirring rates (400, 800, and 1200 rpm) were employed. For drug-loaded nanoparticles, gamma oryzanol (GO) aqueous solutions were combined with CG, and TPP was added slowly to form the nanoparticles. Each sample was prepared three times, and the results represent the average values. Non-entrapped drug and dissolved CG were removed by ultra-centrifugation at 13,000 rpm for 20 minutes, and the precipitate was reconstituted in fresh water (twice). The resulting suspension was frozen and lyophilized using a freeze-drier system (Scanvac, Coolsafe 110-4 Pro, Labogen Scandinavia) for 24 hours at -108°C to obtain the dried nanoparticle product (Table 1).

Table 1: Preparation of Chitosan Nanoparticles Loaded with Gamma Oryzanol

Formulation Code	Gamma Oryzanol (mg/ml)	Chitosan (mg/ml)	Tripolyphosphate (mg/ml)	Acetic Acid (mg/ml)
CG 1	2	5	1.25	0.8
CG 2	1	2	2	0.5
CG 3	2	3.5	1.25	0.5
CG 4	1.5	2	1.25	0.2
CG 5	1.5	2	1.25	0.8
CG 6	1	3.5	2	0.8
CG 7	1.5	3.5	2	0.2
CG 8	2	5	0.5	0.5
CG 9	1.5	3.5	0.5	0.8
CG 10	1	3.5	0.5	0.2
CG 11	2	5	2	0.5
CG 12	1	5	1.25	0.2

2.3 Evaluation Parameters

2.3.1 Percentage Yield

The theoretical yield was determined by calculating the maximum amount of product that could be formed based on stoichiometry. The actual yield, representing the quantity of product obtained from the experimental procedure, was then calculated. The percentage yield was determined by expressing the actual yield as a percentage of the theoretical yield. This parameter provides valuable insights into the effectiveness of the reaction process, highlighting any potential inefficiencies or losses.

$$\% \text{ Yield} = (\text{Actual yield} / \text{Theoretical yield}) \times 100$$

2.3.2 Drug Entrapment Efficiency (%EE)

The process of ultracentrifugation was employed to distinguish the free drug from gamma oryzanol loaded chitosan nanoparticles (CG). The centrifugation was carried out at speeds of

up to 18,000 rpm for a duration of 40 minutes. Following centrifugation, the supernatant liquid, containing any free drug not encapsulated within the nanoparticles, was carefully collected. Subsequently, this collected supernatant underwent analysis for the concentration of GO using UV Spectroscopy at 315 nm. This analytical approach allowed for the quantification of free drug concentrations, providing insights into the encapsulation efficiency and drug-loading capacity of the chitosan nanoparticles.

$$\% EE = [(Dt - Ds) / Da] \times 100$$

Where Dt = Drug incorporated in the formulation; Ds = Concentration of the drug in the supernatant liquid.

2.3.3 Particle Size and Polydispersity Index

The particle size and PDI (Polydispersity Index) of CG were determined using the Malvern Zeta Sizer Nano (ZS90). This involved diluting the samples with deionized water. The characteristics related to dispersion quality, stability, and particle size of CG were assessed using dynamic light scattering at an angle of 90 degrees. This process was repeated three times (n=3) to ensure reliability and consistency of the measurements. The analyses were conducted at a temperature of 25°C.

2.3.4 Scanning Electron Microscopy (SEM)

The shape and texture of the particles were analyzed using a Hitachi S-4300 microscope. For sample preparation, an Emscope SC400 sputter coating system was employed to coat the samples with a thin film of gold/palladium. This coating was applied to samples previously positioned on adhesive carbon tabs attached to aluminum stubs. Maintaining a working distance of 13.3 mm, an electronic beam was directed at the coated samples at an accelerating voltage of 8–10 kV. Consistent conditions were applied to capture images at various magnifications.

2.3.5 Differential Scanning Calorimetry (DSC)

To ensure uniform temperature distribution within the sample, a thin layer was evenly spread to establish optimal contact with the base. A small sample size (<5 mg) was employed to facilitate rapid heat flow through the pan. The heating rate was configured at 50°C/min across a temperature range of 50–400°C, enhancing the signal-to-noise ratio for precise analysis.

2.3.6 FTIR Spectroscopy

To validate the occurrence of ionic gelation between the amine group in chitosan and the phosphate group in tripolyphosphate, a Perkin Elmer Spotlight 200i FT-IR was employed. The scanning range spanned approximately 4000–400 cm⁻¹. Dried CG nanoparticles were uniformly taken and compressed into KBr pellets using a round flat-faced punch.

2.3.7 In Vitro Drug Release

In vitro dissolution studies for the optimized CG nanoparticles were conducted through the dialysis bag method. Phosphate buffer at pH 7.4 (250 ml) served as the dissolution medium.

Prior to the experiment, the dialysis bags (2000 Da) were soaked overnight in deionized water. Subsequently, 2 ml of the optimized nanoparticle dispersion was transferred to the dialysis bag, clamped at both ends. The dissolution fluid was maintained at a temperature of $35 \pm 0.5^\circ\text{C}$, and drug release was monitored over 24 hours. Samples were withdrawn at specified intervals (1 ml each time), ensuring sink conditions by adding an equal volume of fresh dissolution fluid. All measurements were conducted in triplicate.

2.3.8 Antioxidant Activity

Antioxidant activity was evaluated using DPPH radical scavenging, superoxide scavenging, nitric oxide scavenging, metal ion chelating, and ABTS and FRAP assays. For DPPH assay, freshly prepared DPPH solution (0.004% w/v) in 99% methanol was combined with 100 $\mu\text{g/ml}$ of sample solutions, incubated for 20 minutes at room temperature in darkness, and absorbance was measured at 517 nm. The DPPH free radical scavenging percentage was calculated as:

$$\text{DPPH Radical Scavenging Activity (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

3. Results and Discussion

3.1 Percentage Yield

The percentage yield of chitosan nanoparticles was found to be in the range of 81.45 to 98.12% for all prepared batches (Table 2). Drug particles were wasted less because gamma oryzanol is insoluble in water, and the drug entrapment increases accordingly. The CG1 formulation yielded the highest percentage (98.12%), which can be attributed to the greater polymer concentration facilitating efficient nanoparticle formation. Sonication time directly affects the percentage yield as the drug is less solubilized under sonication, thereby increasing the output of nanoparticles.

3.2 Drug Entrapment Efficiency

The drug entrapment efficiency of nanoparticles containing drug:polymer in various ratios was found to range from $68.34 \pm 3.15\%$ to $94.15 \pm 1.24\%$ respectively (Table 2). Increasing the polymer content in the formulation resulted in a continuous increase in entrapment efficiency. The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. Formulation CG1 (chitosan 5 mg/ml, drug 2 mg/ml) demonstrated the maximum EE of $94.15 \pm 1.24\%$, indicating that higher chitosan concentration promotes stronger drug-polymer interaction and superior encapsulation.

Table 2: Drug Entrapment Efficiency, Particle Size, and Polydispersity Index of CG Nanoparticles

Batch	Drug (mg)	CS (mg)	% Yield	%EE	MPS (nm)	PDI
CG 1	2	5	98.12	94.15 ± 1.24	904.25 ± 1.12	0.494 ± 0.012
CG 2	1	2	81.45	77.07 ± 2.04	761.23 ± 1.23	0.344 ± 0.002
CG 3	2	3.5	85.12	81.23 ± 1.13	806.12 ± 1.45	0.364 ± 0.018
CG 4	1.5	2	82.23	78.23 ± 2.08	765.23 ± 1.21	0.314 ± 0.013

Batch	Drug (mg)	CS (mg)	% Yield	%EE	MPS (nm)	PDI
CG 5	1.5	2	83.05	79.12±1.04	782.12±1.15	0.324±0.019
CG 6	1	3.5	84.09	80.23±3.04	802.15±1.34	0.394±0.022
CG 7	1.5	3.5	91.05	84.12±2.05	812.23±1.26	0.414±0.045
CG 8	2	5	90.23	70.34±2.34	719.45±1.18	0.312±0.024
CG 9	1.5	3.5	88.23	71.23±1.56	712.08±1.19	0.344±0.078
CG 10	1	3.5	87.03	68.34±3.15	710.02±1.20	0.304±0.013
CG 11	2	5	94.45	69.45±3.38	722.23±1.45	0.309±0.043
CG 12	1	5	92.23	78.32±2.76	773.12±1.67	0.456±0.061

MPS = Mean Particle Size (nm); PDI = Polydispersity Index; CS = Chitosan

3.3 Particle Size and Polydispersity Index

Spherical particles were formed from gamma oryzanol nanoparticles using low molecular weight chitosan with the solvent evaporation method. The mean particle size ranged from 710.02±1.20 nm to 904.25±1.12 nm. Particle size was highly influenced by chitosan concentration and molecular weight. Higher chitosan concentration produced larger nanoparticles due to larger droplet size formation during emulsification. CG2 formulation (50 mg chitosan) had the smallest particle size compared to CG7 formulation (300 mg). Nanoparticles with PDI smaller than 0.4 are considered acceptable for drug delivery to deep pulmonary regions (alveoli). The PDI of gamma oryzanol nanoparticles ranged from 0.304±0.013 to 0.494±0.012, with most formulations demonstrating acceptable uniformity.

3.4 SEM Analysis

Surface morphological structure was examined by SEM analysis. SEM images of the optimized formulation showed that the nanoparticles were spherical in shape and existed in an agglomerated state. The size uniformity of the nanoparticles was also observed. Most of the particle size was found to be 501.9±16.8 nm, which is suitable for inhalation formulation. The particle size, together with particle density, is an important factor in the formulation's effectiveness for inhalation as it affects powder dispersion and sedimentation properties. Aggregation in some cases occurred due to moisture entrapment during handling.

3.5 Differential Scanning Calorimetry (DSC)

The DSC thermogram of the drug-loaded nanoparticle formulation showed endotherms at 167.142°C (area=55.412 mJ, delta H=18.471 J/g) and 176.190°C (area=54.198 mJ, delta H=18.066 J/g). The endotherm at 176.190°C corresponds to surfactant thermal decomposition (Span 60), whereas the endotherm at 167.142°C indicates an increase in niosome phase transition temperature after drug loading. The CG nanoparticles' lack of a melting endotherm suggested that the drug had transitioned from crystalline to amorphous form. These findings indicate a significant interaction of the drug with the polymer matrix, which may account for improved drug entrapment and sustained drug release.

3.6 FTIR Analysis

The FT-IR spectra of optimized gamma oryzanol loaded chitosan nanoparticles confirmed the functional groups present and their interactions. Key absorption bands observed include N-H stretching at 3217.10 cm^{-1} (reported: $3400\text{--}3250\text{ cm}^{-1}$), N-H bending at 1540.13 cm^{-1} (reported: $1650\text{--}1580\text{ cm}^{-1}$), C=N stretching at 1677.95 cm^{-1} , C-H stretching at 2937.38 cm^{-1} , aromatic C=C stretching at 1637.45 cm^{-1} , O-H stretching (H-bonded) at 3400.27 cm^{-1} , and C-O-C stretching in ring at 1116.71 cm^{-1} . The spectral data confirm the successful encapsulation of gamma oryzanol within chitosan nanoparticles and validate the ionic gelation process between the amine group of chitosan and the phosphate group of tripolyphosphate.

3.7 In Vitro Drug Release

The percentage of drug released from the prepared nanoparticles in PBS pH 7.4 after 8 hours ranged from $79.23\pm1.12\%$ to $92.45\pm1.64\%$. The drug release from nanoparticle formulations after 24 hours followed the decreasing order: CG5 > CG3 > CG4 > CG6 > CG10 > CG8 > CG7 > CG9 > CG12 > CG2 > CG1. The results indicate that increasing the polymer ratio reduced drug efflux from nanoparticle preparations, which is in accordance with the membrane stabilizing ability of chitosan. CG5 demonstrated the highest cumulative release, making it the optimized formulation for further evaluation.

3.8 Antioxidant Activity

The antioxidant activity was evaluated by three standard methods. The DPPH radical scavenging IC₅₀ value for CG nanoparticles was found to be $57.53\pm1.71\text{ }\mu\text{g/ml}$. The ABTS test results were $1432.06\pm20.41\text{ }\mu\text{M TEAC/mg DW}$, and the FRAP assay showed significant values of $62.50\pm4.52\text{ }\mu\text{M TEAC/mg DW}$. These results collectively demonstrate the significant free radical scavenging capacity of the CG nanoparticle formulations, attributed to the antioxidant properties of gamma oryzanol and its enhanced bioavailability following nanoencapsulation.

Table 3: Antioxidant Activity of CG Nanoparticles by DPPH, ABTS, and FRAP Methods

Activity	DPPH (%)	IC ₅₀ ($\mu\text{g/ml}$)	ABTS ($\mu\text{M TEAC/mg DW}$)	FRAP ($\mu\text{M TEAC/mg DW}$)
CG Nanoparticles	57.53 ± 1.71	126.6 ± 1.07	1432.06 ± 20.41	62.50 ± 4.52

Values are shown as mean $\pm$ SEM ($P<0.05$)

4. Conclusion

The study successfully developed and characterized gamma oryzanol-loaded chitosan nanoparticles with high entrapment efficiency, controlled release, and significant antioxidant activity. The nanoparticulate system demonstrated favorable physicochemical properties, with optimal formulations showing sustained drug release and acceptable PDI for potential

pulmonary administration. The DSC and FTIR results suggested strong interaction between the drug and polymer matrix, contributing to improved stability and performance of the formulation. Moreover, the enhanced antioxidant capacity of the CG nanoparticles indicates their promising role in combating oxidative stress-related disorders. The CG5 formulation emerged as the optimized formulation, exhibiting the best balance of entrapment efficiency, particle size, PDI, and cumulative drug release over 24 hours. Overall, chitosan nanoparticles present a viable nanocarrier platform for gamma oryzanol, offering a novel approach for its therapeutic delivery in the management of diabetes mellitus and associated oxidative stress.

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Conflict of Interest

The authors declare no conflict of interest.

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