Research Paper

Formulation and Evaluation of Insulin-Loaded Chitosan Nanoparticles for Oral Delivery in Diabetes Mellitus

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that requires continuous insulin administration for effective glycemic control. However, conventional subcutaneous insulin therapy is associated with poor patient compliance, discomfort, and variable pharmacokinetic profiles. The present study aimed to formulate and evaluate insulin-loaded chitosan nanoparticles as a novel oral delivery system for improving insulin stability and achieving sustained drug release. Insulin-loaded chitosan nanoparticles were prepared by the ionic gelation method using chitosan and sodium tripolyphosphate (TPP). The prepared formulations were characterized for particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, morphology, thermal behavior, and in vitro drug release. Among the developed formulations, F4 demonstrated optimum characteristics with a particle size of 301.5 ± 9.8 nm, zeta potential of +31.4 ± 1.4 mV, entrapment efficiency of 86.9 ± 2.3%, and drug loading of 18.5 ± 0.9%. SEM analysis revealed spherical nanoparticles with smooth surface morphology, while FTIR and DSC studies confirmed successful drug encapsulation and compatibility between insulin and formulation excipients. The optimized formulation exhibited sustained drug release with 91.8% cumulative release over 24 h. Release kinetic analysis indicated diffusion-controlled drug release behavior. Stability studies demonstrated satisfactory physicochemical stability of the formulation during storage. Overall, the developed insulin-loaded chitosan nanoparticles showed promising characteristics for oral insulin delivery and may offer a potential alternative to conventional insulin administration for the management of diabetes mellitus.

Keywords: Diabetes mellitus, Insulin-loaded nanoparticles, Chitosan, Oral insulin delivery, Ionic gelation, Sustained release.

How to cite this article: Subashri S, Janani K, Kanishka P, Natchathira Boopathy R, Dhamodharan V, Sudhakar Kothandan. Formulation and Evaluation of Insulin-Loaded Chitosan Nanoparticles for Oral Delivery in Diabetes Mellitus. Int J Drug Deliv Technol. 2026;16(55s): 1460-1465. DOI: 10.25258/ijddt.16.55s.152

Source of support: Nil.

Conflict of interest: None.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from inadequate insulin secretion, impaired insulin action, or both. Insulin therapy remains the most effective treatment for patients with type 1 diabetes and advanced type 2 diabetes [1], however, conventional subcutaneous administration is associated with pain, poor patient compliance, and fluctuations in blood glucose levels. Oral insulin delivery has emerged as a promising alternative because it mimics the physiological route of insulin transport through the portal circulation [2]. Nevertheless, the oral delivery of insulin is limited by enzymatic degradation and poor intestinal permeability. Nanoparticle-based delivery systems have gained significant attention for overcoming these barriers by protecting insulin from degradation and enhancing its absorption. Among various polymers, chitosan is widely utilized due to its biocompatibility, biodegradability, mucoadhesive nature, and ability to transiently open epithelial tight junctions [3]. Therefore, the present study aimed to formulate and evaluate insulin-loaded chitosan nanoparticles using the ionic gelation technique in order to develop a stable oral delivery system capable of improving insulin encapsulation and providing sustained drug release for effective diabetes management.

2. Materials and Methods

2.1 Materials

Human recombinant insulin was used as the model antidiabetic drug. Chitosan (medium molecular weight, degree of deacetylation 75–85%) was employed as the polymeric carrier, while sodium tripolyphosphate (TPP) was used as a cross-linking agent. Glacial acetic acid, sodium hydroxide, potassium dihydrogen phosphate, disodium hydrogen phosphate, methanol, and other analytical-grade chemicals were utilized throughout the study. All reagents were of analytical grade and used without further purification. Ultrapure water was used in all experiments.

2.2 Formulation of Insulin-Loaded Chitosan Nanoparticles

Insulin-loaded chitosan nanoparticles were prepared by the ionic gelation method. Chitosan was dissolved in 1% (v/v) acetic acid solution under continuous magnetic stirring to obtain a clear polymeric solution. The pH of the solution was adjusted to 5.5 using 0.1 N sodium hydroxide solution. Insulin was dissolved separately in phosphate buffer and added slowly to the chitosan solution under continuous stirring. An aqueous solution of TPP was prepared separately and added dropwise to the chitosan-insulin mixture. The interaction between positively charged amino groups of chitosan and negatively charged phosphate groups of TPP resulted in the spontaneous formation of nanoparticles. The dispersion was stirred continuously for 60 minutes to ensure complete nanoparticle formation. The resultant suspension was centrifuged at 15,000 rpm for 30 minutes, and the nanoparticles obtained were washed with distilled water and freeze-dried using mannitol as a cryoprotectant. Five formulations containing different concentrations of chitosan and TPP were prepared as shown in Table 1 [4-6].

Table 1. Composition of Insulin-Loaded Chitosan Nanoparticle Formulations

Ingredients	F1	F2	F3	F4	F5
Insulin (mg)	50	50	50	50	50
Chitosan (% w/v)	0.10	0.20	0.30	0.40	0.50
TPP (% w/v)	0.05	0.10	0.15	0.20	0.25
Acetic Acid (% v/v)	1.0	1.0	1.0	1.0	1.0
Mannitol (% w/v)	2	2	2	2	2
Distilled Water (mL)	100	100	100	100	100

2.3 Characterization of Insulin-Loaded Chitosan Nanoparticles

2.3.1 Particle Size and Polydispersity Index

The average particle size and polydispersity index (PDI) of the prepared formulations were determined using dynamic light scattering (DLS). The nanoparticle suspension was diluted appropriately with distilled water before analysis. Measurements were carried out in triplicate and expressed as mean ± standard deviation [7].

2.3.2 Zeta Potential Analysis

The surface charge of nanoparticles was determined using a zeta potential analyzer. The zeta potential values were measured to evaluate the stability of the nanoparticle formulations [8].

2.3.3 Morphological Evaluation

The surface morphology and shape of the nanoparticles were examined using scanning electron microscopy (SEM). Samples were mounted on aluminum stubs, sputter-coated with gold, and observed under suitable magnification [9].

2.3.4 Determination of Entrapment Efficiency

The prepared nanoparticle suspension was centrifuged at 15,000 rpm for 30 minutes. The amount of free insulin present in the supernatant was quantified spectrophotometrically. Entrapment efficiency was calculated using Equation:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total Insulin} - \text{Free Insulin}}{\text{Total Insulin}} \times 100$$

2.3.5 Drug Loading Capacity

Drug loading capacity was determined by calculating the percentage of insulin entrapped in relation to the total weight of nanoparticles obtained after freeze-drying [10].

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR studies were performed to evaluate possible interactions between insulin and excipients. The spectra of pure insulin, chitosan, TPP, physical mixtures, and optimized nanoparticles were recorded in the range of 4000–400 cm^{-1} [11]

2.5 Differential Scanning Calorimetry (DSC)

Thermal behavior of insulin, excipients, and optimized formulation was evaluated using differential scanning calorimetry. Samples were heated at a controlled rate under a nitrogen atmosphere and thermograms were recorded.

2.6 In Vitro Drug Release Study

The in vitro release profile of insulin from nanoparticles was evaluated using the dialysis membrane diffusion technique. Nanoparticles equivalent to 10 mg of insulin were placed in a dialysis bag and immersed in phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C under continuous stirring. At predetermined time intervals, samples were withdrawn and replaced with an equal volume of fresh medium. The collected samples were analyzed for insulin content and cumulative percentage drug release was calculated [12].

2.7 Release Kinetic Studies

The in vitro release data were fitted to various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine the mechanism of drug release [13].

2.8 Stability Studies

The optimized formulation was subjected to accelerated stability studies according to ICH guidelines. Samples were stored at 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75 ± 5% RH for a period of three months. Particle size, zeta potential, entrapment efficiency, and drug content were evaluated periodically [14, 5].

3. Results and Discussion

3.1 Evaluation of Insulin-Loaded Chitosan Nanoparticles

Five formulations (F1–F5) were prepared using varying concentrations of chitosan and TPP as presented in Table 1. The prepared nanoparticles were evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and drug loading capacity. The results obtained are summarized in Table 2. As shown in Table 2, particle size increased gradually from 182.4 nm for formulation F1 to 378.2 nm for formulation F5. This increase can be attributed to the higher concentration of chitosan, which increased the viscosity of the polymeric solution and promoted the formation of larger nanoparticles. All formulations exhibited PDI values below 0.35, indicating relatively narrow particle size distribution and acceptable homogeneity. The zeta potential values ranged from +21.8 to +34.8 mV, confirming the cationic nature of chitosan nanoparticles. The increase in positive surface charge with increasing chitosan concentration may contribute to improved colloidal stability and enhanced mucoadhesive properties within the gastrointestinal tract. Entrapment efficiency showed a progressive increase from 68.4% to 88.1% as polymer concentration increased. The higher availability of polymeric matrix likely provided additional sites for insulin incorporation, thereby enhancing drug entrapment. Similarly, drug loading capacity increased from 12.6% to 19.2%, indicating efficient encapsulation of insulin within the nanoparticles. Based on the overall characterization results, formulation F4 was selected as the optimized formulation because it exhibited a favorable balance between particle size, entrapment efficiency, and drug loading.

Table 2. Characterization of Insulin-Loaded Chitosan Nanoparticles

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
F1	182.4 ± 6.3	0.312 ± 0.02	+21.8 ± 1.1	68.4 ± 2.5	12.6 ± 0.8
F2	215.7 ± 8.1	0.298 ± 0.03	+24.5 ± 1.3	74.2 ± 2.1	14.3 ± 0.7
F3	248.9 ± 7.6	0.276 ± 0.02	+28.7 ± 1.2	81.5 ± 1.9	16.8 ± 0.6
F4	301.5 ± 9.8	0.291 ± 0.03	+31.4 ± 1.4	86.9 ± 2.3	18.5 ± 0.9
F5	378.2 ± 12.4	0.336 ± 0.04	+34.8 ± 1.6	88.1 ± 2.6	19.2 ± 1.0

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of pure insulin exhibited characteristic absorption bands at 3292 cm^{-1} corresponding to N–H stretching vibrations, 1654 cm^{-1} corresponding to amide I (C=O stretching), and 1542 cm^{-1} corresponding to amide II vibrations. These peaks confirmed the structural integrity of insulin. Chitosan displayed a broad absorption band at 3421 cm^{-1} due to overlapping O–H and N–H stretching vibrations. Characteristic peaks at 2876 cm^{-1} and 1591 cm^{-1} corresponded to C–H stretching and amino group vibrations, respectively. The FTIR spectrum of TPP showed characteristic phosphate-related peaks at 1212 cm^{-1}, 1149 cm^{-1}, and 890 cm^{-1}, confirming the presence of phosphate groups responsible for ionic crosslinking. The optimized insulin-loaded chitosan nanoparticles (F4) exhibited characteristic peaks at 3386, 1648, 1539, 1215, and 1146 cm^{-1}. Slight shifts in the amide and phosphate peaks compared with the pure components indicated ionic interaction between chitosan and TPP during nanoparticle formation. However, the major characteristic peaks of insulin remained identifiable, suggesting successful encapsulation of insulin without significant chemical degradation or incompatibility. These observations confirmed the formation of a stable chitosan–TPP nanoparticulate system and demonstrated compatibility between insulin and formulation excipients (Figure 1).

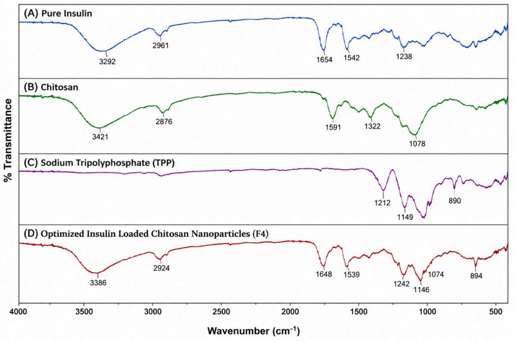

Figure 1. FTIR spectra of (A) Pure Insulin, (B) Chitosan, (C) Sodium Tripolyphosphate (TPP), and (D) Optimized Nanoparticles (F4).

3.3 Differential Scanning Calorimetry (DSC)

The DSC thermograms of pure insulin, chitosan, and optimized insulin-loaded chitosan nanoparticles (F4) are shown in Figure 2**.** Pure insulin exhibited a characteristic endothermic peak at 120.8°C, whereas chitosan showed a broad peak at 99.6°C. The optimized formulation displayed a broadened and less intense peak at 112.4°C, indicating successful encapsulation of insulin within the chitosan matrix. The observed shift and reduction in peak intensity suggest molecular dispersion of insulin and good compatibility between the drug and excipients.

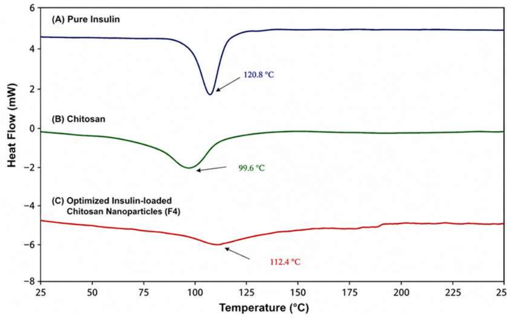

Figure 2. DSC thermogram of pure insulin, chitosan, and optimized nanoparticles (F4).

3.4 Morphological Evaluation

Scanning electron microscopy was employed to examine the morphology of the optimized nanoparticles. SEM micrographs revealed that the nanoparticles were predominantly spherical with smooth surfaces and minimal aggregation. The spherical morphology observed is considered favorable for oral delivery applications because it facilitates uniform drug distribution and may enhance interaction with the intestinal mucosa. The absence of significant aggregation further confirmed the stability of the nanoparticle system. The SEM micrograph of the optimized formulation (Figure 3) demonstrated spherical nanoparticles with smooth surfaces and negligible aggregation.

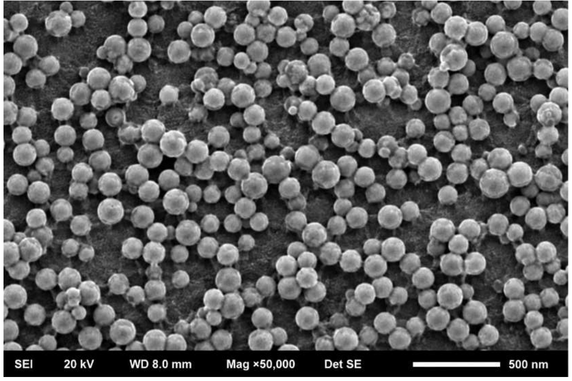

Figure 3. SEM Micrograph of Optimized Nanoparticles

3.5 In Vitro Drug Release Study

The cumulative percentage drug release from formulations F1–F5 was evaluated over a period of 24 hours. The release profiles are presented in Table 3. The release profiles demonstrated a biphasic release pattern characterized by an initial burst release followed by a prolonged release phase. The initial release may be attributed to insulin adsorbed on the nanoparticle surface, while the subsequent controlled release phase resulted from diffusion of insulin through the hydrated polymeric network. An increase in chitosan concentration produced a slower release rate due to the formation of a denser polymeric matrix. Formulation F4 achieved approximately 91.8% cumulative release after 24 hours, indicating effective sustained-release characteristics suitable for oral insulin delivery.

Table 3. Cumulative Percentage Drug Release of Insulin-Loaded Chitosan Nanoparticles

Time (h)	F1	F2	F3	F4	F5
1	18.5	15.8	12.6	10.4	8.7
2	31.2	28.5	24.8	20.6	17.5
4	49.6	46.8	42.5	38.4	34.9
6	64.5	60.2	56.8	52.6	48.7
8	76.4	72.5	69.2	65.8	61.9
12	88.3	85.7	82.9	79.4	75.6
24	97.6	95.2	93.4	91.8	89.5

3.6 Release Kinetic Analysis

The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. The optimized formulation exhibited the highest correlation coefficient with the Higuchi model, indicating that drug release was predominantly diffusion-controlled. The Korsmeyer–Peppas release exponent suggested anomalous transport behavior involving both diffusion and polymer relaxation mechanisms. These findings support the sustained-release characteristics observed during dissolution studies.

3.7 Stability Studies

The optimized formulation (F4) was subjected to stability testing under accelerated and room-temperature storage conditions for three months. The results are presented in Table 4. Only minor variations were observed in particle size, zeta potential, entrapment efficiency, and drug content throughout the storage period. The optimized formulation retained its physicochemical properties without evidence of significant degradation or instability. These findings indicate satisfactory stability of the insulin-loaded chitosan nanoparticles under the investigated storage conditions.

Table 4. Stability Study of Optimized Formulation (F4)

Parameter	Initial	1 Month	2 Months	3 Months
Particle Size (nm)	301.5 ± 9.8	304.2 ± 8.7	308.6 ± 9.3	312.4 ± 10.1

Zeta Potential (mV)	+31.4 ± 1.4	+30.9 ± 1.2	+30.4 ± 1.5	+29.8 ± 1.3
Entrapment Efficiency (%)	86.9 ± 2.3	85.8 ± 2.1	84.9 ± 2.4	84.1 ± 2.2
Drug Content (%)	99.2 ± 1.1	98.4 ± 1.2	97.8 ± 1.4	96.9 ± 1.3

4. Conclusion

The present study successfully formulated and evaluated insulin-loaded chitosan nanoparticles using the ionic gelation technique for potential oral delivery of insulin. The developed nanoparticulate system exhibited favorable physicochemical properties, including nanosized particle dimensions, narrow particle size distribution, positive zeta potential, high entrapment efficiency, and adequate drug loading capacity. Characterization studies confirmed the successful incorporation of insulin into the chitosan matrix without significant drug–excipient incompatibility. SEM analysis revealed predominantly spherical nanoparticles with smooth surface morphology, while FTIR and DSC investigations demonstrated the stability and integrity of the encapsulated insulin. The optimized formulation exhibited a sustained release profile over 24 h, indicating the ability of the chitosan matrix to control insulin release and potentially reduce dosing frequency. Furthermore, the formulation maintained acceptable stability during storage, suggesting its suitability for pharmaceutical development. The mucoadhesive and permeation-enhancing properties of chitosan may contribute to improved intestinal absorption and enhanced oral bioavailability of insulin. Overall, the findings indicate that insulin-loaded chitosan nanoparticles represent a promising oral drug delivery platform capable of overcoming several limitations associated with conventional subcutaneous insulin therapy. Such a system has the potential to improve patient compliance, provide sustained therapeutic effects, and offer a more convenient approach for diabetes management. However, further in vivo pharmacokinetic, pharmacodynamic, and clinical studies are required to establish the long-term efficacy, safety, and translational potential of this nanoparticulate delivery system.

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