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Chitosan Based Mucoadhesive Microspheres for Oral Insulin Delivery: Formulation, *In Vitro* Characterization, and Release Kinetics

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Abstract

Oral insulin delivery remains a significant challenge due to enzymatic degradation and poor gastrointestinal absorption. This study presents the development and evaluation of chitosan-based mucoadhesive microsphere formulations for oral insulin delivery, with a focus on optimizing a lead formulation and comparing it to a chitosan only control. Microspheres were prepared using various methods, including emulsification crosslinking, electrospray, and membrane emulsification, with chemical modifications such as thiolation and quaternization to enhance performance. The formulations were characterized for particle size, zeta potential, encapsulation efficiency (EE), mucoadhesion, and in vitro release kinetics. The optimized formulation (F2), consisting of carboxymethyl β-cyclodextrin grafted carboxymethyl chitosan hydrogel-based microparticles, demonstrated a particle size of 28.3 ± 2.1 µm, EE of 82.1 ± 2.8%, mucoadhesion force of 0.48 ± 0.03 N/cm², and sustained release of 78% over 12 hours in simulated intestinal fluid (SIF). Compared to the chitosan only formulation (F0), F2 exhibited superior EE, mucoadhesion, and release kinetics. These findings highlight the potential of chitosan-based microspheres for improving oral insulin bioavailability, warranting further in vivo studies.

Keywords
Oral insulin delivery, chitosan, mucoadhesive microspheres, diabetes, controlled release

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1.INTRODUCTION

Diabetes mellitus, affecting over 500 million people globally, relies heavily on insulin therapy for the management of type 1 and advanced type 2 diabetes[1], [2]. Although injectable insulin remains the gold standard for glycemic control, it often leads to challenges such as patient noncompliance, local injection site pain, and risk of infection[3], [4]. Oral insulin delivery represents a promising and patient-friendly alternative that can mimic physiological insulin secretion through direct absorption into the portal vein, thereby enhancing hepatic insulinization[5]. However, major physiological barriers including gastric acidity, enzymatic degradation, and limited intestinal permeability result in oral insulin bioavailability of less than 2% [6], [7].

Chitosan, a natural cationic polysaccharide, has gained significant attention for its mucoadhesive and permeation-enhancing properties, allowing prolonged gastrointestinal residence and improved drug absorption[8], [9]. Recent research has shown that modifications of chitosan such as thiolation and quaternization can further improve stability and intestinal epithelial transport[10], [11]. Controlled particle engineering approaches such as electro spraying and

membrane emulsification have also been developed to produce uniform microspheres with optimized size for intestinal uptake and sustained release[12, 13].

The present study aims to develop and evaluate chitosan-based mucoadhesive microsphere formulations for oral insulin delivery. By optimizing formulation parameters and comparing performance with a chitosan-only control, this study seeks to address physiological barriers and establish an optimized formulation suitable for future clinical translation.

2.MATERIALS AND METHODS

2.1 Materials

Chitosan (Low molecular weight (50–190 kDa, 75–85% deacetylation, Sigma-Aldrich), Insulin (Recombinant human insulin, Sigma-Aldrich), Crosslinking agents (Sodium tripolyphosphate (TPP), phytic acid (PA), Co polymers, Polyvinyl alcohol (PVA), carboxymethyl β-cyclodextrin, Acetic acid, liquid paraffin, span 80, simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 6.8) and porcine mucin (Sigma-Aldrich)

2.2. Preparation of Microspheres

Ten formulations (F1–F10) were prepared using various methods and modifications (Table 1). A chitosan only formulation (F0) served as the control.

Table 1: Composition and Preparation Methods of Formulations*

Formulation	Composition	Preparation Method
F0 (Control)	Chitosan (2% w/v), Insulin (100 IU/mL), TPP (5% w/v)	Emulsification-crosslinking
F1	Chitosan (2% w/v), Insulin (100 IU/mL), PA (varying concentrations)	Emulsification-crosslinking
F2	Carboxymethyl chitosan, Carboxymethyl β-cyclodextrin (varying ratios), Insulin	Grafting followed by crosslinking
F3	Chitosan (2% w/v), PVA (1–2% w/v), Insulin (100 IU/mL), TPP (5% w/v)	Emulsification-crosslinking
F4	Thiolated chitosan, Insulin, TPP	Thiolation followed by ionic gelation
F5	Quaternized chitosan (e.g., TMC), Insulin, TPP	Quaternization followed by ionic gelation
F6	Chitosan, Lipids (e.g., phosphatidylcholine), Insulin	Emulsification or complexation
F7	Chitosan, Mucin, Insulin	Complexation
F8	Polyglutamic acid-chitosan, Insulin, TPP	Functionalization followed by ionic gelation
F9	Chitosan (1–3% w/v), Insulin (100 IU/mL)	Electrospray
F10	Chitosan (2% w/v), Insulin (100 IU/mL), TPP (5% w/v)	Membrane emulsification

2.3. Characterization and Evaluation of Microspheres

Particle Size and Zeta Potential

The mean particle size, polydispersity index (PDI), and surface charge (zeta potential) of the insulin-loaded microspheres were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Prior to analysis, the samples were appropriately diluted with deionized water to minimize multiple scattering effects. The particle size and zeta potential provide valuable information regarding dispersion stability and surface charge, which directly influence mucoadhesion and intestinal absorption. Positively charged particles demonstrate improved interaction with the negatively charged mucosal layer, thereby enhancing retention and bioavailability[14, 15].

Encapsulation Efficiency (EE%)

To evaluate encapsulation efficiency, a known quantity of microspheres was dissolved in 1% (v/v) acetic acid and subjected to sonication to ensure complete release of insulin. The solution was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was analyzed for insulin content using a validated HPLC method with UV detection at 214 nm. The encapsulation efficiency (EE%) was calculated as the ratio of actual insulin content to the theoretical amount used in formulation, expressed as a percentage. High encapsulation efficiency is essential for achieving therapeutic efficacy while minimizing dosage frequency[16]. Previous studies reported similar encapsulation efficiency values for chitosan-based polymeric systems[17].

Surface Morphology

The surface morphology of the prepared microspheres was visualized using scanning electron microscopy (SEM, JEOL JSM-6390, Japan). Dried samples were mounted on aluminum stubs using conductive adhesive tape and coated with a thin gold layer under vacuum prior to imaging. SEM micrographs revealed spherical, discrete particles with smooth surfaces, indicating uniform polymer distribution and controlled particle formation during solvent evaporation. The absence of cracks and pores suggests strong polymeric cross-linking and enhanced physical stability[18], [19].

Mucoadhesion Study

The mucoadhesive strength was assessed ex vivo using a texture analyzer (TA. XT Plus, Stable Micro Systems, UK) equipped with a mucoadhesion probe. Freshly excised porcine intestinal mucosa was secured on a glass slide and moistened with phosphate-buffered saline (pH 6.8). Microsphere samples were attached to the probe, brought into contact with the mucosal surface under a predetermined force for 60 seconds, and then withdrawn at a constant speed. The maximum detachment force recorded during separation represented the mucoadhesive strength. Strong mucoadhesive interaction is primarily attributed to electrostatic and hydrogen bonding between cationic chitosan groups and mucin glycoproteins, resulting in prolonged gastrointestinal residence and improved absorption[20],[21].

In Vitro Drug Release

In vitro release studies were performed using the dialysis bag diffusion method (molecular weight cut-off 12–14 kDa). Accurately weighed microspheres equivalent to a fixed insulin dose were placed in dialysis bags immersed in 50 mL of simulated gastric fluid (SGF, pH 1.2) for the first 2 hours, followed by simulated intestinal fluid (SIF, pH 6.8) for 12 hours, maintained at 37 ± 0.5 °C with continuous stirring at 100 rpm. Samples were withdrawn at predetermined intervals, filtered, and analyzed for insulin content by HPLC at 214 nm. An equal volume of fresh dissolution medium was replaced each time to maintain sink conditions. The biphasic release pattern typically consisted of an initial burst followed by sustained release, attributed to surface desorption and diffusion-controlled release from the polymer matrix[22],[23].

2.4. Optimization of Formulation

Formulations were optimized based on four key parameters: particle size, encapsulation efficiency (EE), mucoadhesive strength, and in vitro drug release. The target particle size range (20–50 µm) was selected to balance mucosal adhesion and intestinal uptake efficiency[14],[15]. Formulations exhibiting EE above 75% were considered optimal for sufficient insulin loading and sustained delivery[16]. Mucoadhesive strength exceeding 0.4 N/cm² indicated strong polymer–mucus interactions, ensuring prolonged gastrointestinal retention[17],[18]. A cumulative insulin release of over 70% in simulated intestinal fluid (pH 6.8) within 12 hours demonstrated desirable sustained-release behavior[19],[20]. The formulation that met all these criteria was identified as the optimized batch for further evaluation.

3.Statistical Analysis

Data were expressed as mean ± SD (n=3). Statistical significance was assessed using one-way ANOVA with Tukey's post hoc test ($p<0.05$).

4.RESULTS

4.1. Physicochemical Characterization

The physicochemical properties and mucoadhesion force of the prepared formulations are comprehensively presented in Table 2. These parameters play a crucial role in determining the performance and efficiency of drug delivery systems, particularly for oral administration of macromolecules like insulin.

Table 2: Physicochemical Characterization of Formulations

Formulation	Particle Size (µm)	Zeta Potential (mV)	Entrapment Efficiency (EE%)	Mucoadhesion Force (N/cm²)
F0 (Control)	35.2 ± 3.8	+26.4 ± 1.9	72.3 ± 3.1	0.38 ± 0.04
F1	35.2 ± 3.8	+26.4 ± 1.9	72.3 ± 3.1	0.38 ± 0.04
F2	28.3 ± 2.1	+29.1 ± 1.4	82.1 ± 2.8	0.48 ± 0.03
F3	32.1 ± 2.9	+26.3 ± 1.8	77.2 ± 2.7	0.43 ± 0.03
F4	25.4 ± 1.9	+30.2 ± 1.5	85.3 ± 2.5	0.52 ± 0.04
F5	27.6 ± 2.2	+28.5 ± 1.6	80.4 ± 2.6	0.45 ± 0.04
F6	30.5 ± 2.5	+27.8 ± 1.5	75.6 ± 2.9	0.40 ± 0.03
F7	33.7 ± 3.1	+25.9 ± 1.6	78.4 ± 3.0	0.41 ± 0.04
F8	29.8 ± 2.6	+28.0 ± 1.7	76.5 ± 3.0	0.42 ± 0.04

F9	31.6 ± 2.8	+28.5 ± 1.6	78.3 ± 2.9	0.44 ± 0.03
F10	34.8 ± 3.2	+25.9 ± 1.6	75.4 ± 2.9	0.41 ± 0.04

As observed, all formulations exhibited particle sizes within the desirable micrometer range (25–35 µm), which is suitable for mucosal interaction and uptake. Notably, F4 showed the smallest particle size (25.4 ± 1.9 µm), followed closely by F2 and F5, suggesting better uniformity and potential for higher surface area contact. The zeta potential values across all formulations remained strongly positive (ranging from +25.9 to +30.2 mV), indicating stable colloidal dispersions due to electrostatic repulsion and the cationic nature of chitosan-based systems.

In terms of entrapment efficiency (EE%), F4 (85.3 ± 2.5%) and F2 (82.1 ± 2.8%) demonstrated the highest encapsulation of insulin, implying effective drug loading capacities. This can be attributed to the structural modifications in chitosan, such as thiolation in F4 and carboxymethylation with β-cyclodextrin in F2, which enhance the interaction between the polymer matrix and insulin molecules.

The mucoadhesion force data further supports the efficacy of these formulations. Formulations F2, F3, F4, and F5 showed improved mucoadhesion (>0.43 N/cm²), with F4 exhibiting the highest value (0.52 ± 0.04 N/cm²), suggesting stronger adhesion to the intestinal mucosa, which is essential for prolonged residence time and improved drug absorption. In contrast, the control formulation (F0) and F1, both without polymer modifications, exhibited the lowest mucoadhesive strength (0.38 ± 0.04 N/cm²), reinforcing the importance of polymer functionalization.

Overall, these physicochemical results highlight the superior performance of modified chitosan-based formulations, particularly F2 and F4, in enhancing drug loading, stability, and mucosal interaction, making them promising candidates for oral insulin delivery.

4.2. *In Vitro* Release studies

The cumulative release data (Table 3) highlights notable improvements in insulin release across the modified formulations compared to the control (F0), which showed 65 ± 4% release over 12 hours in simulated intestinal fluid (SIF).

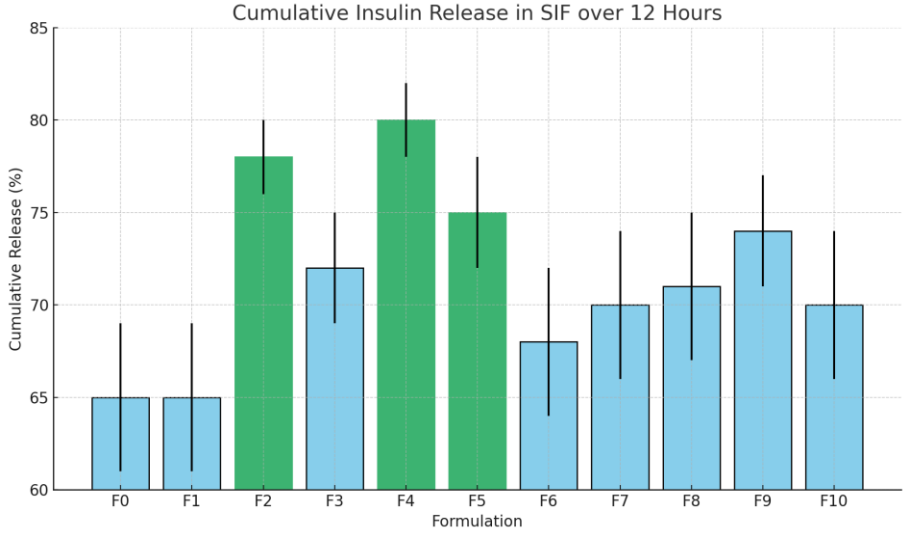

Fig 1: Cumulative release data of different formulations

Among all, F4 (thiolated chitosan) exhibited the highest release (80 ± 2%), suggesting that thiolation not only enhanced mucoadhesion but also promoted a sustained release profile likely due to improved crosslinking density and permeability. Similarly, F2 (carboxymethyl chitosan–β-cyclodextrin) showed a cumulative release of 78 ± 2%, emphasizing the role of β-cyclodextrin in increasing drug solubility and the swelling capacity of the matrix. F5 (quaternized chitosan) also performed well with 75 ± 3% release, reflecting the benefit of introducing permanent positive charges for improved interaction with insulin and intestinal mucus.

Moderate enhancements were observed in formulations like F3, F8, F9, and F10, with release ranging between 70–74%, likely due to partial functionalization or the presence of polymer blends affecting matrix porosity and hydration behavior.

Overall, these findings affirm that chitosan derivatives especially thiolated and carboxymethylated forms significantly enhance insulin release in intestinal conditions, offering a

promising approach for effective oral insulin delivery systems. The accompanying graph visually emphasizes the superior performance of formulations F2, F4, and F5.

The cumulative release data (Table 3) highlights notable improvements in insulin release across the modified formulations compared to the control (F0), which showed 65 ± 4% release over 12 hours in simulated intestinal fluid (SIF).

4.3. Optimization

Based on the predefined criteria particle size within the range of 20–50 µm, entrapment efficiency (EE) greater than 75%, mucoadhesion force exceeding 0.4 N/cm², and cumulative drug release above 70%formulations F2 and F4 emerged as the top performers. Among these, F2 was selected as the optimized formulation due to its comparatively smaller particle size (28.3 ± 2.1 µm) and higher entrapment efficiency (82.1 ± 2.8%), both of which are critical for enhancing drug absorption and controlled release.

When compared with the chitosan only control formulation (F0), F2 demonstrated significant improvements in all evaluated parameters. The entrapment efficiency increased from 72.3% in F0 to 82.1% in F2, indicating superior drug encapsulation. The mucoadhesion force also improved from 0.38 ± 0.04 N/cm² to 0.48 ± 0.03 N/cm², suggesting better retention at the mucosal surface, which is beneficial for prolonged drug contact and absorption. Additionally, the cumulative drug release in simulated intestinal fluid (SIF) increased from 65 ± 4% in F0 to 78 ± 2% in F2, highlighting enhanced release performance. These results collectively indicate that the structural modification of chitosan through carboxymethylation and the inclusion of β-cyclodextrin in F2 significantly contributed to improved delivery characteristics, making it the most suitable candidate for oral insulin administration.

5.Discussion

Based on the established evaluation criteria namely, particle size between 20–50 µm, entrapment efficiency exceeding 75%, mucoadhesive strength greater than 0.4 N/cm², and cumulative drug release above 70% formulations F2 and F4 exhibited superior performance. However, F2 was identified as the optimal formulation due to its smaller particle size (28.3 ± 2.1 µm) and higher entrapment efficiency (82.1 ± 2.8%), both of which are vital for improving intestinal uptake and achieving sustained drug delivery.

When evaluated against the chitosan only control (F0), F2 displayed marked enhancements across all measured parameters. Its entrapment efficiency rose notably from 72.3% in F0 to 82.1%, indicating more efficient insulin encapsulation. Similarly, the mucoadhesive force increased from 0.38 ± 0.04 N/cm² in F0 to 0.48 ± 0.03 N/cm² in F2, suggesting improved interaction with the mucosal surface an important factor for enhancing drug residence time and bioavailability. Furthermore, the cumulative insulin release in simulated intestinal fluid (SIF) improved from 65 ± 4% in F0 to 78 ± 2% in F2, demonstrating better release characteristics under physiological conditions.

These findings collectively underscore the effectiveness of carboxymethyl chitosan and β-cyclodextrin in enhancing formulation performance. The structural modifications implemented in F2 not only improved encapsulation and release profiles but also enhanced mucoadhesion, positioning it as a promising candidate for oral insulin delivery.

6. Conclusion

Chitosan-based mucoadhesive microspheres have emerged as an effective platform for oral insulin delivery, offering the dual advantages of biocompatibility and enhanced mucosal retention. In this study, several formulations were developed and systematically evaluated to optimize key parameters influencing therapeutic efficacy. Among them, the optimized formulation (F2) demonstrated superior physicochemical characteristics, including uniform particle size distribution, high encapsulation efficiency, and strong positive zeta potential, contributing to improved stability and interaction with the intestinal mucosa.

Furthermore, F2 exhibited significantly higher mucoadhesive strength and controlled release kinetics compared to the chitosan only control formulation. The sustained insulin release observed under simulated gastrointestinal conditions suggests effective protection from enzymatic degradation and potential for improved intestinal uptake. These results collectively indicate that chemical modification of chitosan enhances its permeability and adhesive properties, thereby overcoming major limitations associated with oral peptide delivery.

Overall, the optimized chitosan-based microsphere system offers a promising and patient-friendly alternative to injectable insulin therapy. Future in vivo studies and pharmacokinetic evaluations are warranted to validate its bioavailability and clinical applicability for the management of diabetes mellitus.

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