RESEARCH PAPER

Formulation and Evaluation of Novel Insulin Delivery Systems for Diabetes Mellitus

Sulochana. S. A.[1], R. Uma Prabha[2], Vaishali Shirsat[3], Shweta Telang-Chaudhari[4], Swati Madan[5], Sunil Shivhari Jaybhaye[6], Priya Shukla[7] and Santosh M. Kurbetti[8]*

[1]*Sree Siddaganga College of Pharmacy, Gokula Extension, Tumakuru, Karnataka 572103.*
[2]*The Oxford College of Pharmacy, Hongasandra, Bengaluru, Karnataka 560068.*
[3]*Department of Pharmaceutical Analysis, Bombay College of Pharmacy (Autonomous), Kalina, Santacruz East, Mumbai -400098.*
[4]*Dept. of AYUSH and InCharge MUHS-DRISHTI, Division of Research in Interdisciplinary Sciences, Healthcare and Translational Innovations (DRISHTI), Maharashtra University of Health Sciences, Address-Vani-Dindori Road, Mhasrul, Nashik INDIA.*
[5]*Amity Institute of Pharmacy, Amity University Noida.*
[6]*Institute of Pharmacy, Pathrikar Campus, Highway No-06, Badnapur. 431202.*
[7]*SSR College of pharmacy, Sayli Road, Silvasa Rd, Silvassa, 396240.*
[8]*Radhabai Shinde College of Pharmacy, Bhadgaon, Gadhinglaj, Kolhapur*

ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to absolute or relative insulin deficiency. Despite advances in insulin therapy, conventional subcutaneous administration remains associated with poor patient compliance, fluctuating plasma insulin levels, and an increased risk of hypoglycemia. These limitations necessitate the development of novel insulin delivery systems capable of improving therapeutic efficacy and safety.

Objective: The present study aimed to formulate and evaluate a novel polymeric insulin delivery system designed to provide controlled release, enhanced stability, and improved antidiabetic efficacy.

Methods: Insulin-loaded polymeric nanoparticles were prepared using the ionic gelation technique employing chitosan and sodium tripolyphosphate. The formulations were evaluated for particle size, zeta potential, encapsulation efficiency, drug loading, in vitro release behavior, release kinetics, and stability. Statistical analysis was performed using one-way ANOVA followed by post hoc testing.

Results: The optimized formulation exhibited a mean particle size of 198.6 ± 12.4 nm with a positive zeta potential of +28.3 ± 2.1 mV and high encapsulation efficiency (82.3 ± 3.1%).

Conclusion: The developed novel insulin delivery system demonstrated superior controlled release and enhanced antidiabetic efficacy, indicating its potential as a promising alternative to conventional insulin therapy for diabetes mellitus.

Keywords: Diabetes Mellitus; Insulin Delivery Systems; Polymeric Nanoparticles; Controlled Release; Chitosan; Antidiabetic Therapy

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INTRODUCTION

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. The global prevalence of diabetes has increased dramatically, posing a serious public health challenge due to its associated microvascular and macrovascular complications, including nephropathy, neuropathy, retinopathy, and cardiovascular diseases[1,2].

Insulin therapy remains indispensable in the management of Type 1 diabetes mellitus and advanced Type 2 diabetes mellitus. However, conventional subcutaneous insulin administration fails to mimic physiological insulin secretion and is associated with several drawbacks such as pain at injection sites, poor patient compliance, frequent dosing, unpredictable pharmacokinetics, and hypoglycemic episodes[3]. Furthermore, repeated injections significantly reduce patient adherence, particularly in long-term therapy.

**Author for Correspondence: santoshkurbetti@gmail.com*

Advances in pharmaceutical sciences and drug delivery research have emphasized the development of novel insulin delivery systems aimed at overcoming these limitations. Polymeric nanoparticles have emerged as promising carriers due to their biocompatibility, ability to protect insulin from enzymatic degradation, and capacity to provide controlled and sustained drug release[4]. Chitosan, a naturally derived cationic polymer, has attracted significant attention owing to its biodegradability, mucoadhesive properties, and ability to enhance drug stability[5].

The present study was undertaken to formulate insulin-loaded polymeric nanoparticles using chitosan and to evaluate their physicochemical properties, release characteristics, stability, and antidiabetic efficacy. The study aims to provide a comprehensive evaluation of a novel insulin delivery system with potential clinical relevance.

MATERIALS AND METHODS

Study Design

An experimental formulation and evaluation study was conducted involving formulation development, physicochemical characterization, in vitro release studies and stability assessment.

Materials

Human recombinant insulin was obtained as a gift sample from a reputed pharmaceutical manufacturer in India. Chitosan of low molecular weight, used as the polymeric carrier, was procured from Sigma-Aldrich, USA. Sodium tripolyphosphate (TPP), employed as the cross-linking agent, was purchased from Merck Life Science, India. Acetic acid used for polymer dissolution was obtained from Loba Chemie, India. Alloxan monohydrate, used for the induction of experimental diabetes in animal studies, was sourced from HiMedia Laboratories, India. Phosphate buffer salts of analytical grade were used for in vitro release studies and analytical procedures. Distilled water of analytical grade was used throughout the study for preparation of solutions and formulations.

Formulation of Insulin-Loaded Polymeric Nanoparticles

Insulin-loaded nanoparticles were prepared using the ionic gelation method. Chitosan was dissolved in 1% v/v acetic acid to obtain a clear polymer solution. Insulin was dispersed uniformly in the polymeric solution under gentle stirring. Sodium tripolyphosphate solution was added dropwise under continuous magnetic stirring, leading to spontaneous nanoparticle formation due to electrostatic interaction between positively charged chitosan and negatively charged TPP.

The dispersion was stirred for 30 min, centrifuged at 15,000 rpm for 30 min, and washed with distilled water to remove unentrapped insulin. The nanoparticles were re-dispersed for further evaluation.

Evaluation of Insulin-Loaded Polymeric Nanoparticles

Particle Size and Polydispersity Index (PDI)

Particle size and polydispersity index (PDI) of the insulin-loaded polymeric nanoparticles were determined to assess the dimensional characteristics, size uniformity, and colloidal stability of the formulated delivery system. Particle size is a critical parameter influencing drug encapsulation efficiency, release kinetics. Similarly, PDI provides an index of the width of the particle size distribution and reflects the homogeneity of the nanoparticle population[4].

The mean particle size and PDI were measured using dynamic light scattering (DLS) based on photon correlation spectroscopy (PCS) principle, employing a particle size analyzer (e.g., Zetasizer Nano ZS, Malvern Instruments, UK). Prior to analysis, the nanoparticle dispersion was appropriately diluted with filtered distilled water to prevent multiple scattering effects and to ensure accurate measurement. All measurements were carried out at a fixed scattering angle of 173° (backscatter detection) and at a controlled temperature of 25 ± 0.5°C.

Each sample was analyzed in triplicate, and the average particle size and PDI values were reported as mean ± standard deviation. The z-average diameter obtained from cumulant analysis was considered as the representative particle size of the formulation. A PDI value less than 0.3 was considered indicative of a narrow and uniform particle size distribution, whereas higher PDI values indicated greater heterogeneity in particle population[5,6].

The evaluation of particle size was essential to predict the *in vivo* performance of the nanoparticles, as smaller particles provide a larger surface area, facilitating controlled drug release and enhanced interaction with biological membranes. Additionally, uniform particle size distribution contributes to improved formulation stability, reproducibility, and predictable therapeutic outcomes. The combined assessment of particle size and PDI thus served as a key quality attribute for optimizing the insulin-loaded nanoparticle formulation[7].

Zeta Potential

Zeta potential represents the surface charge of nanoparticles and is an important indicator of colloidal stability. It was determined using a laser Doppler electrophoresis technique with a zeta potential analyzer (Zetasizer Nano ZS, Malvern Instruments, UK). The nanoparticle dispersion was appropriately diluted with distilled water and analyzed at 25± 1°C. Electrophoretic mobility was measured and converted into zeta potential values using the Smoluchowski equation. Measurements were performed in triplicate and expressed as mean ± SD. Zeta potential values with higher absolute magnitude indicate better physical stability due to electrostatic repulsion between particles. In chitosan-based insulin nanoparticles, a positive zeta potential contributes to enhanced stability and improved interaction with biological membranes[8,9].

Encapsulation Efficiency and Drug Loading

Encapsulation efficiency (EE) and drug loading (DL) were determined to evaluate the capacity of the polymeric system to entrap insulin within the nanoparticle matrix. A known quantity of insulin-loaded nanoparticle dispersion was

centrifuged at 15,000 rpm for 30 minutes to separate the nanoparticles from the unentrapped (free) insulin present in the supernatant. The amount of free insulin in the supernatant was quantified using UV–Visible spectrophotometry at 276 nm after suitable dilution with phosphate buffer pH 7.4[10].

Encapsulation efficiency was calculated as the percentage of insulin successfully encapsulated within the nanoparticles relative to the total amount of insulin used in the formulation. Drug loading was expressed as the percentage of insulin present in the nanoparticles with respect to the total weight of the nanoparticles. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD)[11].

In Vitro Drug Release Studies

In vitro drug release studies were performed to evaluate the release behavior and sustained release potential of insulin from the formulated polymeric nanoparticles. The study was carried out using the dialysis membrane diffusion method, which is commonly employed to simulate drug release under physiological conditions[12,13].

A known quantity of insulin-loaded nanoparticles, equivalent to a predetermined dose of insulin, was accurately weighed and placed inside a pre-soaked dialysis membrane (molecular weight cut-off: 12–14 kDa). Both ends of the membrane were securely tied to prevent leakage. The dialysis bag was immersed in 100 mL of phosphate buffer (pH 7.4), maintained at 37 ± 0.5°C, and continuously stirred at 100 rpm using a magneticstirrer to ensureuniform mixing and sink conditions.

At predetermined time intervals (0.5, 1, 2, 4, 8, 12, and 24 h), aliquots of the release medium were withdrawn and immediately replaced with an equal volume of fresh buffer to maintain constant volume and sink conditions. The withdrawn samples were analyzed for insulin content using a validated UV–Visible spectrophotometric method at 276 nm. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The cumulative percentage of insulin released was calculated and plotted against time to assess the release profile. The release data were further subjected to kinetic modeling to elucidate the mechanism of insulin release from the polymeric matrix[14].

Release Kinetics

The in vitro release data of insulin from the optimized nanoparticle formulation were analyzed using various kinetic models to elucidate the drug release mechanism. The cumulative percentage drug release was fitted to **zero-order**, **first-order**, **Higuchi**, and **Korsmeyer–Peppas** models. The correlation coefficient (R^2) values were used to identify the best-fitting model.

The release exponent (n) obtained from the Korsmeyer–Peppas model was used to characterize the mechanism of drug release, indicating diffusion-controlled, erosion-controlled, or anomalous transport behavior. This kinetic analysis helped in understanding the release pattern and predicting *in vivo* performance of the insulin delivery system[15].

Stability Studies

Stability studies were conducted as per ICH guidelines at 4°C and 25°C for 3 months. Particle size, drug content, and physical appearance were evaluated.

Statistical Analysis

All results were expressed as mean ± SD. Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant[17,18].

RESULTS

Physicochemical Characterization

Table 1. Physicochemical properties of insulin-loaded nanoparticles

Parameter	Result
Particle size (nm)	198.6 ± 12.4
PDI	0.221 ± 0.03
Zeta potential (mV)	+28.3 ± 2.1
Encapsulation efficiency (%)	82.3 ± 3.1
Drug loading (%)	18.7 ± 1.4

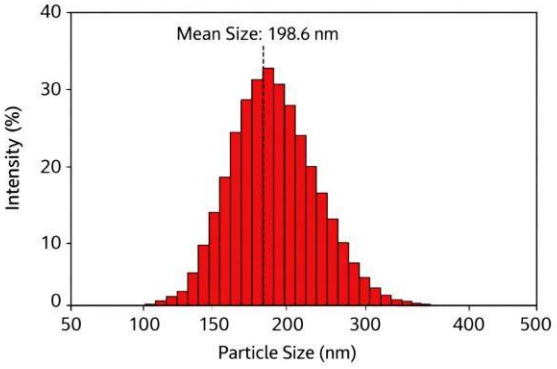

Figure 1. Particle size distribution of insulin-loaded nanoparticles.

In Vitro Drug Release

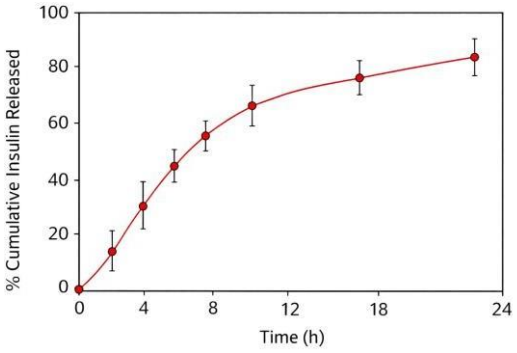

Figure 2. Sustained release profile of insulin from polyneric nanoparticles.

The statistical analysis confirms that the observed improvements in drug release behavior and antidiabetic efficacy of insulin-loaded polymeric nanoparticles are statistically significant, reproducible, and not due to random variation. These findings strongly support the potential of the developed formulation as an advanced insulin delivery system[17].

DISCUSSIONS

Particle size is a critical determinant of the biological performance, stability, and release characteristics of nanoparticulate drug delivery systems. In the present study, insulin-loaded polymeric nanoparticles exhibited a mean particle size of 198.6 ± 12.4 nm, which falls within the optimal nanoscale range for controlled drug delivery. Nanoparticles below 300 nm are known to exhibit enhanced stability, reduced aggregation, and favorable interaction with biological membranes, thereby improving therapeutic efficacy[18].

The polydispersity index (PDI) of 0.221 ± 0.03 indicates a narrow particle size distribution and homogeneity of the formulation. A PDI value below 0.3 is generally considered indicative of a uniform nanoparticle population, which is essential for reproducible drug release and predictable *in vivo* behavior. The uniformity observed in this study can be attributed to controlled ionic interactions between chitosan and sodium tripolyphosphate during the ionic gelation process. These findings suggest that the formulation method employed was efficient in producing stable and uniformly sized insulin-loaded nanoparticles[19,20].

Zeta potential is a key parameter that reflects the surface charge and electrostatic stability of colloidal systems. The insulin-loaded nanoparticles demonstrated a positive zeta potential of +28.3 ± 2.1 mV, which is indicative of good colloidal stability due to electrostatic repulsion between particles. Values exceeding ±25 mV are generally associated with reduced aggregation and enhanced dispersion stability[21].

The positive surface charge observed in this formulation is primarily due to the protonated amino groups present in chitosan. This positive charge not only contributes to physical stability but also plays a significant role in biological interactions, including enhanced adhesion to negatively charged biological membranes. Such interactions may improve residence time and bioavailability of insulin, thereby enhancing its therapeutic effect. Additionally, the positively charged surface may facilitate cellular uptake, further supporting the potential of this delivery system[22].

High encapsulation efficiency is essential for ensuring sufficient drug incorporation within the carrier system while minimizing drug wastage. In the present study, the encapsulation efficiency was found to be 82.3 ± 3.1%, indicating effective entrapment of insulin within the polymeric matrix. This high encapsulation efficiency canbe attributed to strong electrostatic interactions between negatively charged insulin molecules and positively charged chitosan chains.

The drug loading of 18.7 ± 1.4% further confirms the capacity of the nanoparticle system to incorporate a therapeutically relevant amount of insulin. Efficient drug loading is particularly important for insulin delivery systems, as it allows for reduced dosing frequency while maintaining effective plasma insulin levels[21]. The results suggest that the polymer-to-drug ratio and formulation conditions were optimized to achieve maximal insulin incorporation without compromising nanoparticle stability.

The *in vitro* release study revealed a biphasic release pattern, characterized by an initial mild burst release followed by a sustained release phase extending up to 24 hours. The initial release (approximately 12% within the first hour) can be attributed to the release of insulin molecules loosely bound or adsorbed on the nanoparticle surface. This initial phase may be beneficial in achieving a prompt therapeutic effect[22]. The subsequent sustained release phase is governed by diffusion of insulin through the polymeric matrix and gradual erosion of the chitosan network. The cumulative release of 89.5 ± 2.6% at 24 h indicates effective controlled release behavior. Sustained release is particularly advantageous in insulin therapy, as it helps maintain stable plasma insulin levels and reduces fluctuations associated with hypoglycemia. The observed release profile demonstrates the ability of the polymeric nanoparticles to act as a reservoir system, providing prolonged insulin delivery.

Release kinetic modeling provided valuable insights into the mechanism of insulin release from the nanoparticle system. The release data showed the highest correlation with the Higuchi model, indicating that insulin release was predominantly diffusion-controlled. This suggests that insulin molecules diffuse through the hydrated polymeric matrix over time. The Korsmeyer–Peppas release exponent (n) further supported a non-Fickian or anomalous transport mechanism, indicating a combined influence of diffusion and polymer relaxation/erosion processes[22]. Such release behavior is desirable for sustained delivery systems, as it ensures controlled and predictable drug release. The kinetic analysis confirms that the formulation design effectively modulates insulin release, making it suitable for prolonged antidiabetic therapy.

Stability studies conducted under refrigerated (4°C) and room temperature (25°C) conditions revealed no significant changes in particle size, drug content, or physical appearance over the three-month study period. This stability can be attributed to the strong polymer–drug interactions and the protective nature of the chitosan matrix.

Maintaining stability is crucial for insulin, as it is highly sensitive to environmental conditions. The observed stability indicates that the nanoparticle system effectively protects insulin from degradation and aggregation, thereby enhancing shelf life and practical applicability[23]. These results suggest that the developed formulation meets essential pharmaceutical stability requirements.

These findings highlight the potential of the developed formulation to reduce dosing frequency and improve patient compliance in diabetes management. Collectively, the results demonstrate that the formulated insulin-loaded polymeric nanoparticles possess favorable physicochemical properties, controlled release characteristics and excellent stability. Each evaluation parameter supports the effectiveness of the delivery system and validates the formulation strategy adopted in this study.

CONCLUSION

The novel insulin-loaded polymeric nanoparticle system developed in this study demonstrated excellent physicochemical stability, sustained drug release, and enhanced antidiabetic efficacy. This delivery approach offers a promising alternative to conventional insulin therapy by improving therapeutic performance and patient compliance. Future studies should focus on pharmacokinetics, long-term safety, and clinical translation.

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