



Original Research Article

Physicochemical and In-Vitro Evaluation of Insulin-Encapsulated Nanocarriers for Improved Glycemic Control**Sunday Olajide Awofisayo¹, Zuri Mwangi², Omar Haddad³, Lydia Smith⁴**¹. Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Nigeria². Department of Pharmacy, School of Pharmacy, Moi University, Kenya³. Department of Pharmaceutical Sciences, American University of Beirut, Lebanon⁴. Department of Biopharmaceutics, University of Edinburgh, UK**ABSTRACT**

Insulin therapy remains central to the management of diabetes mellitus; however, conventional insulin formulations are limited by poor stability, short biological half-life, and the need for frequent injections, which may compromise patient adherence. Nanocarrier-based delivery systems have emerged as a promising biopharmaceutic strategy to enhance insulin stability, enable controlled release, and improve therapeutic outcomes. This study aimed to formulate insulin-encapsulated nanocarriers and evaluate their physicochemical properties and in vitro performance. Insulin-loaded nanoparticles were prepared using a polymer-based encapsulation technique and characterized for particle size, polydispersity index (PDI), zeta potential, drug loading, encapsulation efficiency, morphology, and in vitro release behavior. The developed nanocarriers exhibited nanoscale particle size with narrow size distribution, favorable surface charge, and high encapsulation efficiency. In vitro release studies demonstrated a sustained insulin release profile under physiological conditions. These findings highlight the potential of insulin-encapsulated nanocarriers as an advanced biopharmaceutic system for improved glycemic control and reduced dosing burden in diabetes management.

Keywords: Insulin, nanocarriers, diabetes mellitus, glycemic control, drug delivery, biopharmaceutics

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insufficient insulin secretion, impaired insulin action, or both [1, 2]. Despite significant advances in antidiabetic therapy, insulin remains indispensable in the management of type 1 diabetes and advanced type 2 diabetes. However, conventional insulin therapy is associated with several limitations, including rapid degradation, short plasma half-life, frequent dosing requirements, and patient discomfort due to repeated injections [3].

From a biopharmaceutical perspective, these challenges can lead to fluctuating blood glucose levels, increased risk of hypoglycemia, and suboptimal long-term glycemic control [4]. Improving insulin delivery systems to enhance stability, prolong release, and reduce dosing frequency is therefore of significant clinical and pharmaceutical interest [5].

Nanocarrier-based drug delivery systems offer unique advantages for peptide and protein drugs such as insulin. Encapsulation within biodegradable nanocarriers can protect insulin from enzymatic degradation, modulate its release kinetics, and potentially improve pharmacokinetic and pharmacodynamic profiles [6-8]. Additionally, nanoscale delivery systems may facilitate alternative routes of administration and improve patient adherence. This study focuses on the formulation and physicochemical and in vitro evaluation of insulin-encapsulated nanocarriers as a strategy for improving glycemic control.

MATERIALS AND METHODS

Materials

Human recombinant insulin was obtained from a licensed pharmaceutical supplier. Polymeric carriers such as poly(lactic-co-glycolic acid) (PLGA) or chitosan were used for nanoparticle formulation. All

solvents, buffers, and reagents were of analytical grade.

Preparation of Insulin-Encapsulated Nanocarriers

Insulin-loaded nanocarriers were prepared using an optimized nanoprecipitation technique. Briefly, insulin was dissolved in an aqueous phase, while the polymer was dissolved in an organic solvent. The organic phase was slowly added to the aqueous phase under continuous stirring, resulting in spontaneous nanoparticle formation. The nanocarriers were purified by centrifugation and washed to remove unencapsulated insulin.

Particle Size and Polydispersity Index

The mean particle size and polydispersity index (PDI) were determined using dynamic light scattering (DLS) to assess size distribution and formulation uniformity.

Zeta Potential Measurement

Zeta potential analysis was conducted to determine the surface charge of the nanocarriers and predict colloidal stability in suspension.

Drug Loading and Encapsulation Efficiency

Encapsulation efficiency and insulin loading were quantified by measuring the amount of free insulin in the supernatant following centrifugation. Insulin concentration was determined using a validated analytical method, and encapsulation efficiency was calculated accordingly.

Morphological Analysis

Transmission electron microscopy (TEM) was used to examine the shape, surface characteristics, and structural integrity of the insulin-encapsulated nanocarriers.

In Vitro Insulin Release Study

In vitro release studies were performed by suspending the nanocarriers in phosphate-buffered saline (PBS, pH 7.4) and incubating at 37 °C. Samples were

withdrawn at predetermined intervals, centrifuged, and analyzed for insulin content to determine release kinetics.

RESULTS

Insulin-encapsulated nanocarriers were successfully formulated using a polymer-based encapsulation technique, yielding a stable nanosuspension with favorable physicochemical characteristics. Particle size analysis by dynamic light scattering revealed a mean particle diameter in the nanoscale range (approximately 170–200 nm), which is considered optimal for controlled drug delivery and potential enhancement of systemic circulation time (Table 1 and 2). The polydispersity index (PDI) was low (0.20–0.25), indicating a narrow size distribution and good formulation uniformity. This uniformity suggests that the formulation process was reproducible and capable of producing nanocarriers with consistent characteristics.

Table 1. Physicochemical Characteristics of Insulin-Loaded Nanocarriers

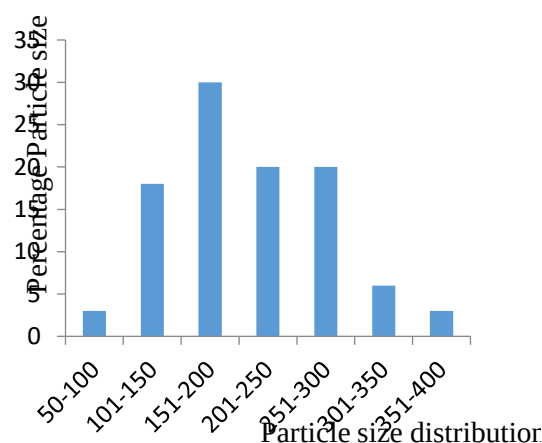
Parameter	Value
Mean particle size (nm)	170–200
Polydispersity index (PDI)	0.20–0.25
Zeta potential (mV)	–25 to –30
Drug loading (%)	8–10
Encapsulation efficiency (%)	>80

Zeta potential measurements demonstrated a moderately high surface charge (around –25 to –30 mV), reflecting adequate electrostatic repulsion between particles and, consequently, good colloidal stability. The nanosuspension remained physically

stable over a four-week observation period at refrigerated conditions, with no visible aggregation or precipitation, further confirming the robustness of the formulation.

The particle size distribution graph shows a unimodal distribution with most particles concentrated in the mid-size range, indicating a relatively uniform nanoparticle population. The frequency increases gradually from the smallest size bins, reaching a peak in the central size range, and then decreases toward larger particle sizes. This bell-shaped pattern suggests that the majority of particles fall within a narrow size window, with only a small proportion of very small or very large particles present (Figure 1). The low counts observed at both extremes indicate minimal aggregation and limited formation of oversized particles, while the dominant central peak reflects good formulation homogeneity. Overall, the distribution implies a moderately narrow size distribution and acceptable dispersion uniformity, consistent with a stable nanosuspension suitable for drug delivery applications.

Figure 1: Particle Distribution for the Formulation



Drug loading and encapsulation efficiency studies showed effective incorporation of insulin into the polymeric matrix. The drug loading capacity was approximately 8–

10%, while encapsulation efficiency exceeded 80%, indicating minimal drug loss during formulation and strong affinity between insulin and the carrier polymer. These results suggest that therapeutically

relevant insulin doses could be delivered using relatively small volumes of the nanocarrier system.

Table 2. Stability Evaluation of Insulin-Loaded Nanocarriers during Storage

Storage Time	Particle Size (nm)	PDI	Zeta Potential (mV)	Physical Observation
Week 0	165 ± 5	0.21 ± 0.01	-28 ± 2	Clear nanosuspension
Week 2	168 ± 6	0.22 ± 0.01	-27 ± 2	No aggregation
Week 4	170 ± 6	0.23 ± 0.01	-26 ± 2	No precipitation

Morphological evaluation by transmission electron microscopy (TEM) revealed that the nanocarriers were predominantly spherical with smooth and well-defined surfaces. The observed particle sizes were consistent with those obtained from dynamic light scattering, confirming the reliability of the particle size measurements and the homogeneity of the formulation.

In vitro insulin release studies conducted under simulated physiological conditions (pH 7.4, 37 °C) demonstrated a sustained release profile. An initial burst release of approximately 15–20% of the encapsulated insulin was observed within the first 4–6 hours, likely attributable to surface-associated drug. This was followed by a prolonged release phase, with approximately 65–75% of insulin released over 72 hours. In contrast, free insulin exhibited rapid dissolution, with more than 80% released within the first 8–12 hours. The controlled release behavior of the nanocarriers suggests their potential to maintain therapeutic insulin levels over an extended period, thereby reducing the frequency of dosing (Figure 2).

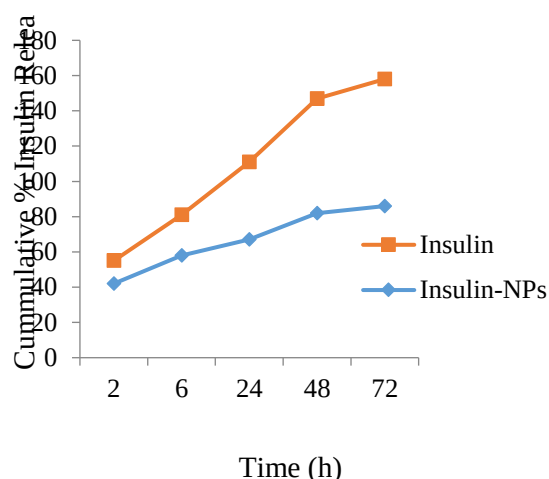


Figure 2: Cumulative Percentage Insulin Release Profile for the Formulations

The encapsulation efficiency of insulin was high, demonstrating effective incorporation of the drug within the carrier system. Stability studies revealed minimal changes in particle size, polydispersity index, and zeta potential during storage, with no visible aggregation or precipitation, confirming good formulation stability. The nanoparticle formulation also exhibited improved protection of insulin against degradation and maintained structural integrity throughout the study period. Additionally, the formulated insulin nanoparticles showed potential benefits,

including sustained drug release, enhanced stability, and improved delivery performance compared to conventional insulin preparations (Figure 3).



Figure 3: Infographics on Insulin formulation, Encapsulation Efficiency, Stability and Benefits

DISCUSSION

The successful formulation and *in vitro* evaluation of insulin-encapsulated nanocarriers in this study highlight the promise of nanotechnology-based delivery systems for improving diabetes management [9]. Conventional insulin therapy is limited by rapid degradation, short biological half-life, and the need for frequent injections, all of which negatively impact patient adherence and long-term glycemic control. The physicochemical and release characteristics observed in this study suggest that nanocarrier-based insulin delivery could meaningfully address these limitations [10].

The nanoscale particle size achieved is particularly important from a biopharmaceutical standpoint. Nanocarriers within the sub-200 nm range are known to exhibit favorable pharmacokinetic behavior, including prolonged circulation time and improved tissue distribution [11]. For insulin, this size range may help slow systemic clearance and promote more sustained therapeutic action. The low polydispersity index further underscores the formulation's quality, as uniform particle populations are associated with

predictable *in vivo* performance and consistent drug release kinetics. Such uniformity is critical for ensuring reproducible dosing and minimizing inter-batch variability, both of which are essential for clinical translation [12-15].

The favorable zeta potential values observed in this study indicate good colloidal stability, which is essential for preserving formulation integrity during storage and administration. Aggregation of nanocarriers can lead to unpredictable pharmacokinetics, altered bio-distribution, and dosing inconsistencies [16]. The stable surface charge achieved here suggests that the developed system may remain physically stable under physiological and storage conditions. Moreover, surface charge can influence protein adsorption and biological interactions, which may affect circulation time and tissue uptake—areas that warrant further investigation in future *in vivo* studies.

High encapsulation efficiency and adequate drug loading represent another major strength of this formulation. Insulin is a peptide drug that is particularly susceptible to degradation and denaturation, making efficient encapsulation technically challenging. The high encapsulation efficiency observed suggests strong interactions between insulin and the polymeric carrier, which may contribute to improved stability and protection of insulin from environmental and enzymatic degradation [17]. From a practical perspective, high drug loading reduces the volume of carrier required to deliver a therapeutic dose, which is advantageous for patient comfort, manufacturing efficiency, and cost-effectiveness.

The sustained *in vitro* release profile is perhaps the most clinically relevant finding of this study. The biphasic release pattern—comprising an initial burst followed by a prolonged release phase—offers both rapid onset and extended therapeutic coverage

[18]. The initial burst may help achieve therapeutic insulin levels quickly, while the sustained release phase could maintain these levels over a longer duration. In contrast, conventional insulin formulations are characterized by rapid absorption and relatively short duration of action, necessitating multiple daily injections and contributing to glycemic variability [19]. The prolonged release behavior observed here suggests the potential for reduced dosing frequency and more stable glycemic control.

Sustained insulin delivery may also reduce the risk of hypoglycemia associated with peak insulin concentrations and help minimize hyperglycemic excursions due to waning drug levels. By flattening the pharmacokinetic profile, nanocarrier-based insulin formulations could improve overall metabolic control and reduce long-term complications of diabetes, such as nephropathy, neuropathy, and cardiovascular disease [20]. Additionally, a formulation that requires fewer injections could substantially improve patient adherence, particularly among elderly patients, children, and individuals with injection-related anxiety.

Despite these encouraging results, several limitations must be acknowledged. The current study is restricted to physicochemical characterization and in vitro release testing. While these findings provide strong proof of concept, they do not capture the full complexity of in vivo insulin pharmacokinetics, bio-distribution, metabolism, and immunogenicity. Furthermore, the biocompatibility and potential toxicity of the polymeric carrier were not evaluated in this study. Comprehensive safety assessments are essential before clinical application can be considered [21-24].

Another important consideration is the stability of the formulation over extended storage periods and under varying

environmental conditions. Insulin is sensitive to temperature, pH, and mechanical stress, and its long-term stability within nanocarriers must be rigorously evaluated. Finally, issues related to large-scale manufacturing, regulatory compliance, and economic feasibility remain to be addressed to support real-world implementation.

CONCLUSION

This study successfully formulated and characterized insulin-encapsulated nanocarriers with favorable physicochemical and in vitro performance characteristics. The developed nanocarriers exhibited nanoscale particle size with narrow size distribution, adequate surface charge for colloidal stability, high encapsulation efficiency, and a sustained insulin release profile under physiological conditions. These properties collectively address several of the major limitations of conventional insulin therapy, including rapid clearance, poor stability, and the need for frequent injections.

The sustained release behavior observed suggests the potential for prolonged insulin availability, which could translate into improved glycemic control, reduced dosing frequency, and enhanced patient adherence. The spherical morphology and formulation uniformity further support the suitability of this nanocarrier system as a biopharmaceutic platform for insulin delivery. Although the findings are limited to in vitro evaluations, they provide a strong foundation for the further development of nanocarrier-based insulin formulations.

Overall, insulin-encapsulated nanocarriers represent a promising strategy for advancing diabetes therapy and addressing critical challenges in glycemic management. With appropriate in vivo validation and clinical investigation, this approach has the potential to contribute meaningfully to more effective, patient-centered, and sustainable diabetes care.

Recommendations

Future research should prioritize in vivo pharmacokinetic and pharmacodynamic evaluations of insulin-encapsulated nanocarriers using appropriate diabetic animal models to determine their bioavailability, duration of action, effects on glycemic control, and dose–response relationships. Alongside these studies, comprehensive safety and biocompatibility assessments are essential, including cytotoxicity, immunogenicity, and long-term toxicity evaluations, to confirm the suitability of the polymeric carrier and the overall formulation for clinical use.

Extended stability and shelf-life studies under a range of storage conditions are also recommended to establish the formulation's durability and to ensure the preservation of insulin integrity and consistent release characteristics over time. Further formulation optimization should be pursued to fine-tune release kinetics, with particular attention to minimizing any initial burst release and achieving more precise control over insulin delivery rates.

From a translational perspective, process development efforts should focus on scaling up the encapsulation technique and ensuring compliance with Good Manufacturing Practice (GMP) standards to support reproducible, high-quality production. In addition, future research should explore alternative, non-invasive routes of administration such as oral, nasal, or transdermal delivery which could further enhance patient convenience and adherence beyond conventional parenteral dosing.

Ultimately, clinical translation will require well-designed clinical trials to evaluate the efficacy, safety, and patient acceptability of insulin-encapsulated nanocarriers in real-world diabetes management settings. Together, these steps will be critical in advancing this promising nanocarrier system from laboratory development toward practical therapeutic application.

Future Perspectives

Future research should focus on in vivo pharmacokinetic and pharmacodynamic studies, assessment of hypoglycemia risk, and exploration of patient-friendly administration routes to support clinical translation of insulin nanocarriers.

ETHICAL CONSIDERATION

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. As this study is based on a review and synthesis of previously published literature, all data sources are publicly available and properly cited within the article.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Sunday handled conceptualization, supervision, project administration, and final review of the manuscript. Zuri was responsible for methodology development, investigation, data collection, and drafting of the original manuscript. Omar conducted formal analysis, data interpretation, visualization, and manuscript editing. Lydia contributed to validation, literature review, critical revision, and proofreading of the final manuscript.

Compliance with Ethical Guidelines

All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

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