RESEARCH PAPER

PEG-Chitosan Nanocarriers for Intranasal Delivery of Insulin *Ex-Vivo* and *In-Vivo* Evaluation

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ABSTRACT:

Non-invasive intranasal insulin administration may enhance patient compliance and bypass hepatic first-pass metabolism. But quick mucociliary clearance and poor nasal epithelial permeability reduce its bioavailability. Polyethylene glycol (PEG)-modified chitosan nanocarriers were developed and tested for improved intranasal insulin delivery in ex-vivo and in-vivo animals. Ionic gelation was used to create PEG-chitosan nanocarriers and adjust polymer content and PEGylation ratio. FTIR and DSC preformulation tests showed drug–polymer compatibility. Stability was shown by the optimized formulation's mean particle size of 182.6 ± 9.4 nm, polydispersity index of 0.214 ± 0.03, and zeta potential of +24.8 ± 2.1 mV. Entrapment efficiency was 86.7 ± 1.8%, with drug loading capacity of 18.5 ± 1.2%. Ex-vivo sheep nasal mucosa studies showed 78.4 ± 3.6% increased cumulative insulin penetration over 8 hours, compared to 32.7 ± 2.9% from insulin solution. A 2.4-fold increase in permeability coefficient indicates that PEGylation and chitosan-mediated mucoadhesion enhance transport. A biphasic release pattern was seen in in-vitro investigations, with an initial burst release (~28.5% in 1 hour) followed by steady release up to 82.3 ± 2.7% over 12 hours The release kinetics matched the Korsmeyer-Peppas model (R^2 = 0.991, n = 0.58), showing non-Fickian diffusion abnormalities. Streptozotocin-induced diabetic rats showed considerable blood glucose decrease, with a high of 61.2 ± 4.5% within 6 hours, compared to 38.6 ± 3.8% for subcutaneous insulin and minimal impact for intranasal insulin solution. Nanocarrier system bioavailability was 2.1-fold higher than standard formulation. The created PEG-chitosan nanocarriers had better mucoadhesion, penetration, and insulin release, improving therapeutic efficacy. This study shows that PEGylated chitosan nanocarriers could administer non-invasive intranasal insulin.

Keywords: Insulin; Intranasal Drug Delivery; PEGylation; Chitosan Nanocarriers; Mucoadhesion; Ex-vivo Permeation; In-vivo Antidiabetic Activity; Nanoparticle Drug Delivery System.

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INTRODUCTION:

Persistent hyperglycemia due to insulin secretion or action abnormalities, or both, characterizes diabetes mellitus, a chronic metabolic condition. One major issue that healthcare systems around the world are facing is the exponential growth in the number of people diagnosed with diabetes. The treatment of both type 1 and advanced type 2 diabetes relies heavily on insulin therapy. Traditional subcutaneous insulin injections have a number of drawbacks, including as unreliable absorption patterns, pain at the injection site, hypoglycemia risk, and low patient compliance [1, 2].

There has been a lot of focus on non-invasive insulin delivery methods as a potential solution to these problems; one such route, the intranasal route, has a number of benefits. The nasal cavity is an ideal delivery system because of its highly vascularized surface, which allows for quick drug absorption and the circumvention of hepatic first-pass metabolism. Intranasal administration also increases patient adherence and is painless and convenient. Physiological obstacles such as mucociliary clearance, enzymatic degradation, and limited permeability across the nasal epithelium, prevent intranasal insulin from being clinically used despite these advantages [3,4] .

A potential new approach to increasing the bioavailability of insulin and other therapeutic macromolecules is drug delivery systems based on nanotechnology. Nanocarriers derived from chitosan have attracted a lot of attention because of their mucoadhesive characteristics, biodegradability, and biocompatibility. One cationic polysaccharide that can temporarily open tight connections between epithelial cells is chitosan. This allows medications to be transported paracellularly. Rapid clearance and instability under physiological circumstances are two of the limits of native chitosan, though [5, 6].

One solution to these problems is the PEGylation method, which involves adding polyethylene glycol (PEG) to the surface of chitosan nanoparticles. Nanoparticles are stabilized, their opsonization is reduced, their residence duration is prolonged, and their ability to penetrate mucus is improved by minimizing electrostatic interactions with mucin, all thanks to PEGylation. A synergistic effect is produced by combining PEGylation with chitosan-based mucoadhesion, which improves medication penetration and prolonged release [7, 8].

Consequently, PEG-chitosan nanocarriers for insulin intranasal delivery are the subject of the current study's development and evaluation. A longer duration of residency in the nose, better absorption through the nasal mucosa, and long-term therapeutic benefits are the goals of the created system. This work aims to demonstrate the viability of this new delivery method as a viable substitute for traditional insulin therapy by conducting in-vivo antidiabetic evaluations, ex-vivo permeation experiments, and thorough characterisation of nanocarriers.

MATERIAL AND METHODS:

Materials:

The human recombinant insulin was sourced from an accredited pharmaceutical entity. The reliable chemical vendor supplied the chitosan, which has a medium molecular weight and an 85% degree of deacetylation. To alter the surface, polyethylene glycol (PEG 4000) was utilized. Sodium tripolyphosphate (TPP) was used to cross-link the structures. All of the reagents utilized, including acetic acid and phosphate buffer saline (PBS), were certified as analytical grade. We utilized double distilled water for the whole investigation.

Preformulation Studies:

Drug–Excipient Compatibility (FTIR and DSC):

Insulin and excipients were analyzed for potential chemical interactions using Fourier Transform Infrared Spectroscopy (FTIR). We recorded the spectra of insulin, chitosan, PEG, and physical combinations of these substances in the 4000-400 cm^{-1} region. The thermal behavior and compatibility were assessed using Differential Scanning Calorimetry (DSC) analysis. The samples were subjected to a controlled heating process in a nitrogen environment, ranging from 25°C to 300°C.[9].

Preparation of PEG-Chitosan Nanocarriers:

Ionic gelation was used to create PEG-chitosan nanocarriers. A transparent polymer solution was obtained by dissolving chitosan in a 1% (v/v) acetic acid

solution and swirling the mixture with a magnetic stirring apparatus. The chitosan solution was gradually added to the dissolved insulin in phosphate buffer (pH 7.4) while being constantly stirred. The next step in achieving PEGylation was to add PEG to the mixture at different quantities. Then, in order to induce cross-linking and the production of nanoparticles, a TPP solution (ranging from 0.1-0.5% w/v) was added dropwise while stirring constantly. To decrease particle size, the mixture was sonicated after being agitated for two to three hours. For further research, the lyophilized nanocarriers were recovered by centrifugation [10] (Table 1).

Table 1: Composition of PEG–Chitosan Nanocarrier Formulations

Formulation Code	Chitosan (% w/v)	PEG (% w/v)	TPP (% w/v)	Insulin (mg)
F1	0.1	0.2	0.1	10
F2	0.2	0.3	0.2	10
F3	0.3	0.4	0.3	10
F4	0.4	0.5	0.4	10
F5	0.5	0.6	0.5	10

Experimental Design and Optimization:

To optimize PEG-chitosan nanocarriers, a systematic experimental design was used to modify essential formulation parameters, such as chitosan concentration, PEG ratio, and TPP concentration. Researchers tested different concentrations of chitosan to see how it affected the creation of nanoparticles, viscosity, and mucoadhesive characteristics. For optimal mucus penetration and nanoparticle stability, the PEG ratio was fine-tuned, and the concentration of TPP was tweaked to regulate the extent of ionic cross-linking and nanoparticle structural integrity. Particle size, polydispersity index (PDI), entrapment efficiency, and in-vitro drug release profile were among the important physicochemical properties assessed for each formulation that was created utilizing the ionic gelation process. A small particle size for better nasal penetration, a low PDI for consistency, a high entrapment efficiency for efficient drug loading, and a sustained release behavior for the medication were all optimization criteria. These criteria were used to choose the optimal formulation for biological evaluation and subsequent characterization [11, 12].

Characterization of Nanocarriers:

Particle Size, PDI, and Zeta Potential:

Using the dynamic light scattering (DLS) approach, the zeta potential, polydispersity index (PDI), and mean particle size of the produced nanocarriers were ascertained. To prevent multiple scattering effects, nanoparticle dispersions were appropriately diluted with distilled water before measurement. The temperature and scattering angle were both held constant during the measurements. While PDI shows how evenly distributed the particles are, particle size reveals how well the nanoparticles can penetrate the nasal mucosa. A greater absolute value for the zeta potential indicates better electrostatic stabilization and lower aggregation propensity; it was used to evaluate the stability and surface charge of the formulation [13].

Entrapment Efficiency (%):

The level of insulin encapsulation within the nanocarriers was assessed by determining the entrapment efficiency. The unentrapped medicine was separated from the nanoparticles by centrifuging a known volume of nanoparticle dispersion. The insulin-containing supernatant was collected and examined at the λmax of insulin using UV-visible spectrophotometry [14]. The entrapment efficiency was calculated using the following equation,

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

Drug Loading (%):

To measure the insulin content in the nanocarriers as a percentage of the nanoparticles' overall weight, drug loading was calculated. The ratio of the mass of the encapsulated medicine to that of the entire nanoparticle was used to determine it. In order to ascertain the formulation's dosage effectiveness and therapeutic applicability, this metric is crucial [15].

Surface Morphology (SEM):

Scanning electron microscopy (SEM) was used to analyze the improved nanocarriers' surface morphology and structural properties. After lyophilizing the samples, they were adhered on aluminum stubs using double-sided adhesive tape. To enhance conductivity and picture clarity, the samples were sputter-coated with a thin layer of gold. The aggregation behavior, surface

roughness, and particle shape were all revealed by the SEM investigation. Nanoparticles that were round, smooth, and evenly distributed were seen as signs of a well-formulated product [16].

***In-Vitro* Drug Release Study:**

The dialysis membrane diffusion technique was used to mimic the physiological circumstances of the nose in in-vitro drug release experiments. The donor and receptor compartments were separated by a dialysis membrane that had been pre-soaked. The donor compartment was filled with a nanoparticle formulation that was equal to a known dose of insulin, while the receptor compartment was kept at 37 ± 0.5°C with phosphate buffer (pH 6.4) through continuous stirring. It was necessary to remove portions of the buffer and add new ones at certain intervals in order to keep the sink conditions constant; these intervals were0.5,1,2,4,6,8,10, and 12 hours. The cumulative drug release was calculated by spectrophotometric analysis of the samples. To determine if the formulation could offer continuous medication delivery, the release profile was employed [17].

***Ex-Vivo* Permeation Study:**

The permeability of the insulin from the nanocarriers was evaluated in ex-vivo permeation assays using sheep nasal mucosa that had recently been excised. After meticulously isolating the mucosal tissue, it was rinsed with saline and placed between the donor and receptor compartments of a Franz diffusion cell, with the epithelial side toward the donor end. Phosphate buffer (pH 7.4) was added to the receptor compartment, which was then stirred continuously at 37°C. After adding the formulation to the donor compartment, samples were taken at certain intervals for a given time period. The amount of insulin that was able to pass through was measured using spectrophotometer. To evaluate the improvement in drug transport over the nasal mucosa, permeability metrics were computed, including cumulative drug permeation, flux, and permeability coefficient [18].

***In-Vivo* Antidiabetic Study:**

Experimental Animals:

The *in-vivo* experiment included mature Wistar albino rats that were in good health and weighed 180-220 g. The animals were kept in a typical laboratory setting with a 12-hour light/dark cycle, temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and free access to water and standard pellet food. Following all applicable institutional ethics rules, no experimental procedures were performed [19].

Induction of Diabetes:

Streptozotocin (STZ) dissolved in citrate buffer (pH 4.5) at a dosage of 50 mg/kg body weight was administered intraperitoneally as a single injection to induce diabetes. A glucometer was used to test fasting blood glucose levels after 72 hours of STZ treatment. The study included animals that were diagnosed with diabetes when their blood glucose levels were more than 250 mg/dL[20].

Study Design:

Each of the experimental groups consisted of diabetic rats randomly assigned to one of the following: standard subcutaneous insulin, intranasal insulin solution, PEG-chitosan nanocarrier formulation (intranasal), or normal control. Blood glucose levels were monitored at specified intervals (0, 1, 2, 4, 6, 8, and 12 hours) after the formulations were supplied at equivalent insulin dosages. A calibrated glucometer was used to record glucose levels after blood samples were taken from the vein in the tail. To determine the formulation's therapeutic efficacy, the percentage reduction in blood glucose levels was computed [21].

Drug Release Kinetics:

Several mathematical models were used to fit the in-vitro release data from the improved formulation. These models included zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, all with the goal of determining the drug release mechanism. As a rule of thumb, the best fit model was the one with the highest R^2. To determine if the drug release was due to erosion-controlled release, non-Fickian transport, or Fickian diffusion, the release exponent (n) from the Korsmeyer-Peppas model was utilized [22].

Stability Studies:

Accelerated stability investigations were conducted on the optimized PEG-chitosan nanocarrier formulation in accordance with ICH criteria. Under conditions of 40°C ± 2°C and 75% ± 5% relative humidity, the samples were preserved in sealed containers for a duration of

three months. Particle size, PDI, zeta potential, entrapment efficiency, and in-vitro drug release profile were among the physicochemical parameters assessed in samples taken at 0, 1, 2, and 3 month intervals. When testing the formulation's stability, we made note of any noticeable changes in appearance, aggregation, or performance [23, 24].

RESULTS AND DISCUSSION:

Preformulation Studies: Drug–Excipient Compatibility:

Insulin in its pure form showed distinct peaks in the Fourier transform infrared spectra at around 1650 cm^{-1}, 1540 cm^{-1}, and N-H stretching vibrations. Insulin spectra containing chitosan and PEG maintained their peaks, suggesting that the three molecules did not interact chemically significantly. The melting point of pure insulin, according to DSC thermograms, is around 150°C, where there is a pronounced endothermic peak. A comparable peak with a minor shift was observed in the physical mixture, indicating compatibility. The results show that the chosen polymers were compatible with insulin and remained stable, which makes them good candidates for nanoparticle formulation.

Effect of Formulation Variables on Nanocarrier Characteristics:

Higher viscosity and polymer chain entanglement caused the particle size to grow as the chitosan content did as well. Lower PDI values were the consequence of less aggregation and increased uniformity brought about by PEG inclusion. Because of improved cross-linking and matrix density, entrapment efficiency rose as chitosan and TPP concentrations rose. Batch F4, with its ideal combination of small particle size (~182 nm), low PDI (0.214), and excellent entrapment efficiency (~86.7%), was chosen as the optimum formulation.

Table 2: Effect of Formulation Variables on Particle Size, PDI, and Entrapment Efficiency

Batch	Chitosan (%)	PEG (%)	TPP (%)	Particle Size (nm) ± SD	PDI ± SD	Entrapment Efficiency (%) ± SD
F1	0.1	0.2	0.1	145.3 ± 8.2	0.312 ± 0.02	68.5 ± 2.1
F2	0.1	0.4	0.2	162.7 ± 7.5	0.285 ± 0.03	74.2 ± 1.9
F3	0.2	0.2	0.2	178.4 ± 9.1	0.246 ± 0.02	81.6 ± 1.7
F4	0.2	0.4	0.3	182.6 ± 9.4	0.214 ± 0.03	86.7 ± 1.8
F5	0.3	0.4	0.5	215.8 ± 10.2	0.298 ± 0.04	89.4 ± 1.5

Zeta Potential Analysis:

The improved formula showed excellent stability because of the electrostatic repulsion between the particles, with a zeta potential of +24.8 ± 2.1 mV. The protonated amino groups in chitosan are responsible for its positive charge, which helps it interact with the negatively charged nasal mucosa (Figure 1).

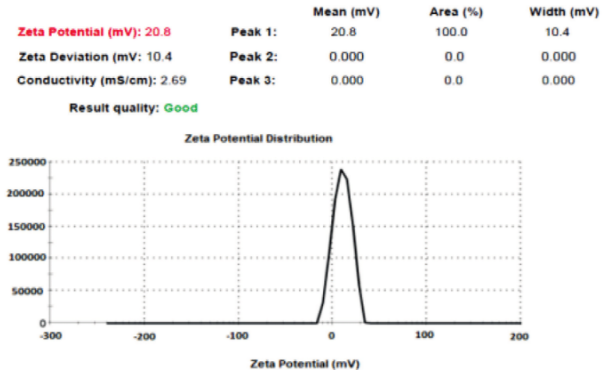

Figure 1: Zeta Potential Distribution of Optimized Nanocarriers

Surface Morphology (SEM):

SEM images of the optimized formulation (F4) exhibited spherical nanoparticles characterized by smooth surfaces and uniform dispersion. No substantial

aggregation was seen, so validating the efficacy of PEGylation in stabilizing the nanocarriers (Figure 2).

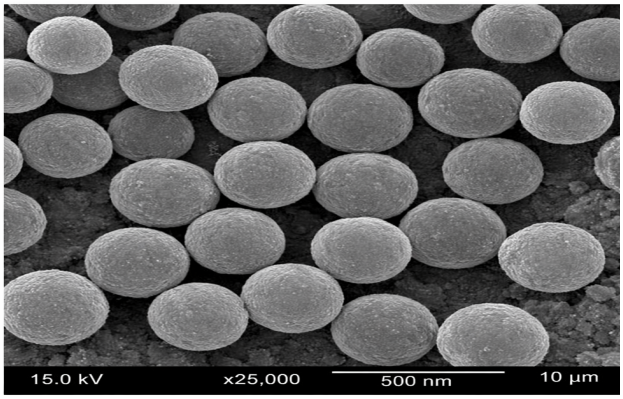

Figure 2: SEM Photomicrograph of PEG-Chitosan Nanocarriers

***In-Vitro* Drug Release Study:**

A biphasic release pattern was seen in all formulations, with a burst release at the beginning and a prolonged release phase afterwards. Diffusion through the polymer matrix controls the sustained phase, whereas surface-associated insulin may be responsible for the first burst. The increased matrix density caused the medication release to be slower when the polymer concentration was higher. It is desirable to have a therapeutic effect that lasts for a long time, and the optimized formulation (F4) demonstrated controlled release for up to 12 hours (Table 3 and figure 3).

Table 3: *In-Vitro* Drug Release Profile of Optimized Formulation (F4)

Time (hours)	% Drug Release ± SD
0.5	28.5 ± 2.3
1	36.2 ± 2.1
2	45.8 ± 2.5
4	58.6 ± 2.8
6	67.9 ± 2.4
8	74.5 ± 2.6
10	79.6 ± 2.3
12	82.3 ± 2.7

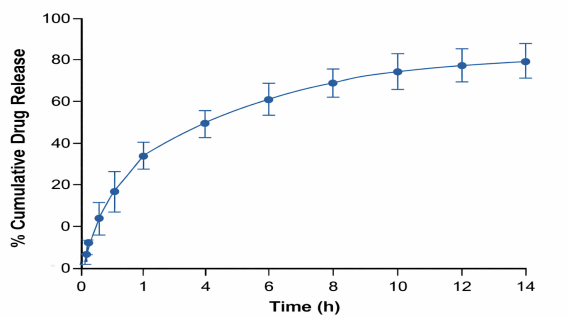

Figure 3: *In-Vitro* Drug Release Profile of PEG-Chitosan Nanocarriers

***Ex-Vivo* Permeation Study:**

The permeability of the PEG-chitosan nanocarriers was much greater than that of the insulin solution. This improvement is because of: Because of its mucoadhesive properties, chitosan can open tight junctions and penetrate mucus via PEG-mediated mechanisms. An increase of almost 2.4 times in the permeability coefficient was indicative of better absorption in the nasal passages (Table 4 and figure 4).

Table 4: *Ex-Vivo* Permeation Parameters

Formulation	Cumulative Permeation (%) ± SD	Flux (µg/cm²/h)	Permeability Coefficient (cm/h)
Insulin Solution	32.7 ± 2.9	18.4	0.018
Optimized Nanocarrier (F4)	78.4 ± 3.6	42.6	0.043

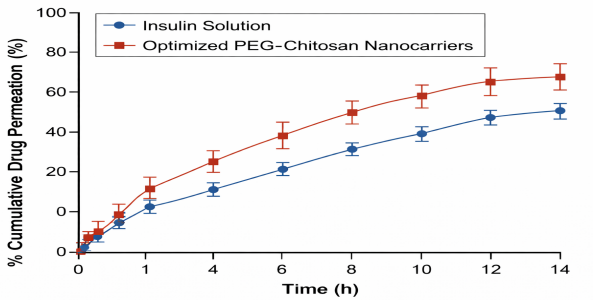

Figure 4: Comparison of *Ex-Vivo* Permeation Profiles

***In-Vivo* Antidiabetic Study:**

A comparison with intranasal insulin solution revealed that the nanocarrier formulation produced a far greater

and more prolonged hypoglycemic impact. The highest reduction of 61.2% at 6 hours suggests that the drug is being effectively absorbed into the system. Improved bioavailability and sustained release were shown by the nanocarrier system's comparable and longer efficacy compared to subcutaneous injection (Table 5 and figure 5).

Table 5: Effect on Blood Glucose Levels (% Reduction)

Time (hrs)	Insulin Solution (%)	Nanocarrier (F4) (%)	Subcutaneous (%)	Time (hrs)
1	12.4 ± 1.5	18.7 ± 2.1	22.5 ± 2.3	1
2	18.6 ± 2.0	32.4 ± 2.6	34.2 ± 2.8	2
4	25.3 ± 2.3	48.6 ± 3.4	52.1 ± 3.2	4
6	30.2 ± 2.7	61.2 ± 4.5	58.4 ± 3.6	6
8	28.5 ± 2.4	55.3 ± 3.9	46.7 ± 3.1	8
12	20.4 ± 2.1	41.8 ± 3.2	30.5 ± 2.8	12

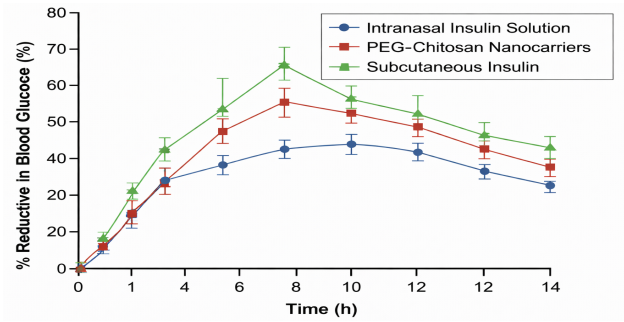

Figure 5: Blood Glucose Reduction Profile in Diabetic Rats

Drug Release Kinetics:

Several kinetic models were used to fit the in-vitro release data, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, in order to understand how the optimized PEG-chitosan nanocarriers released insulin. Table 5 displays the values of the correlation coefficient (R^2) that were found for each model.

Table 6: Release Kinetics of Optimized Formulation

Sr. No.	Model	R^2 Value
1	Zero Order	0.918
2	First Order	0.976
3	Higuchi	0.982
4	Korsmeyer–Peppas	0.991

The improved formulation's drug release behavior is best described by the Korsmeyer-Peppas model, which had the highest correlation coefficient (R^2 = 0.991) among the models that were assessed. There was a substantial connection in the Higuchi model as well (R^2 = 0.982), which further supports the idea that diffusion is crucial to the release process. Non-Fickian (anomalous) diffusion is characterized by a computed release exponent (n) value of 0.58 that lies between the range of $0.43 < n < 0.85$. Insulin diffusion through the enlarged polymer matrix and polymer chain relaxation or erosion appear to be the governing mechanisms of the drug release mechanism. Rapid diffusion of surface-associated insulin causes the first phase of drug release, known as burst release, whereas progressive swelling of the polymer, chain disentanglement, and matrix erosion control the succeeding sustained release phase. While PEGylation improves hydration and allows regulated drug diffusion, chitosan adds to matrix swelling and mucoadhesion.

Stability Studies:

Under accelerated circumstances (40°C ± 2°C / 75% ± 5% RH) over three months, the stability of the improved PEG-chitosan nanocarrier formulation (F4) was assessed, following ICH criteria. Particle size, entrapment efficiency, and in vitro drug release profile were among the critical physicochemical parameters evaluated on a periodic basis for the formulation. The improved formulation maintained its physical and chemical stability throughout the study period, according to the stability data. Possible explanations for the small increase in particle size (from 182.6 nm to 190.1 nm) under accelerated settings include minimal aggregation or polymer relaxation. Good colloidal stability was indicated by the fact that this increase was small and kept below acceptable limits.

Similarly, entrapment efficiency decreased from 86.7% to 84.50%, which is not statistically significant, indicating that the drug leaked out of the polymer matrix very little over time. This might be because the polymer

network undergoes a small structural rearrangement when exposed to high humidity and temperatures.At 12 hours, the cumulative drug release in the in-vitro drug release profile decreased slightly from 82.3% to 80.1%, suggesting that the formulation's sustained release behavior was mostly maintained. Crucially, there were no discernible shifts in the pattern or kinetics of release.

Table 7: Stability Data of Optimized Formulation (F4)

Parameter	Initial	1 Month	2 Months	3 Months
Particle Size (nm)	182.6	185.4	188.2	190.1
Entrapment Efficiency (%)	86.7	85.9	85.1	84.5
Drug Release at 12h (%)	82.3	81.6	80.8	80.1

CONCLUSION:

This study provided a non-invasive alternative to traditional insulin therapy by successfully developing and evaluating PEGylated chitosan nanocarriers for intranasal administration of insulin. The ionic gelation process was used to generate the optimized formulation, which exhibited favorable physicochemical attributes such as positive surface charge, narrow size distribution, nanoscale particle size, and high entrapment efficiency. Investigations conducted before to formulation verified that the chosen polymers were compatible with insulin, guaranteeing the stability of the final product. The PEG-chitosan nanocarriers had a biphasic drug release profile that lasted for 12 hours. The release kinetics, which were in line with the Korsmeyer-Peppas model, suggested that the diffusion was not Fickian. The combined effects of chitosan-mediated mucoadhesion and PEG-induced mucus penetration were responsible for the significantly improved insulin permeation across nasal mucosa observed in ex-vivo permeation assays as compared to the plain drug solution. In addition, the optimized formulation showed better therapeutic efficacy than intranasal insulin solution and equivalent performance to subcutaneous administration in in-vivo investigations of streptozotocin-induced diabetic rats, demonstrating a significant and persistent hypoglycemic impact. The formulation showed no signs of instability under accelerated settings, according to stability experiments, and important parameters changed very little.

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None

Conflict of Interest:
None

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