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Abstract

Introduction: Type 1 Diabetes Mellitus (DM) is a chronic autoimmune disease marked by progressive destruction of pancreatic β -cells. **Aim:** Development and characterization of novel delivery systems for insulin using solid dispersion technique to enhance therapeutic efficacy, patient convenience. **Methods:** Biocompatible polymers such as Hydroxypropyl Methylcellulose (HPMC), Ethyl Cellulose (EC), and Soluplus[®], were employed via solvent evaporation and melt methods. The matrixes were characterized for their physicochemical properties and assessed for *in vivo* anti-diabetic efficacy in alloxanized diabetic mice. **Results:** The solid dispersion batches demonstrated good flow properties, with Hausner's ratios ranging from 1.02 ± 0.01 to 1.07 ± 0.01 and compressibility indices between 2.13 ± 1.01 % and 6.61 ± 0.9 %. Encapsulation efficiencies ranged from 54.21 % to 78.69 %. *In vitro* release studies in simulated gastric and intestinal fluids indicated up to 80 % insulin release within 80 min. Stability studies revealed negligible changes in pH during storage. *In vivo* studies showed a significant reduction in blood glucose levels in treated groups compared to controls. Hematological and hepatic assessments indicated insignificant toxicity. **Conclusion:** These highlight the potential of polymer-based oral insulin formulations as a promising alternative to conventional injection therapies, offering improved glycemic control in type1-DM management.

Keywords: Diabetes mellitus, Insulin, Solid dispersion, Drug delivery system, Blood glucose

Introduction

Diabetes mellitus is a chronic endocrine disorder characterized by persistent hyperglycemia, resulting from impaired insulin secretion, insulin action, or a combination of both. The

condition is associated with significant disturbances in carbohydrate, protein, and lipid metabolism. Globally, the prevalence of diabetes has increased substantially, with 14% of adults aged 18 years and older affected in 2022, compared to 7% in 1990 [1]. In 2021, diabetes was directly responsible for approximately 1.6 million deaths, with nearly 47% of these fatalities occurring in individuals under the age of 70 [2]. Moreover, diabetes and its related complications, such as diabetic nephropathy, accounted for over 2 million deaths in the same year. Elevated blood glucose levels were also implicated in roughly 11% of all cardiovascular-related mortalities [3,4]. Given its escalating incidence and associated morbidity and mortality, diabetes mellitus has been termed the epidemic of the century. Without timely diagnosis, effective treatment, and comprehensive disease management, diabetes-related mortality is projected to continue rising significantly [5,6]. Diabetes mellitus is commonly classified into three major categories: insulin-dependent diabetes mellitus (Type 1), non-insulin-dependent diabetes mellitus (Type 2), and other forms, including gestational diabetes mellitus [6]. Type 1 diabetes mellitus specifically is a chronic autoimmune condition in which inflammation occurs around the pancreatic islets, ultimately resulting in the targeted destruction of β -cells responsible for insulin production. Conversely, Type 2 diabetes mellitus involves a combination of reduced sensitivity to insulin in peripheral tissues and a diminished capacity of the pancreas to secrete insulin effectively [7]. The hormone insulin plays a critical role in the regulation of blood sugar levels. It is secreted by the pancreas, an organ located posterior and inferior to the stomach, and released into the bloodstream where it facilitates the uptake of glucose into cells, thereby reducing circulating blood glucose levels. As blood glucose concentrations decrease, insulin secretion correspondingly diminishes. Type 1 diabetes develops when the immune system—ordinarily responsible for defending the body against pathogenic microorganisms—mistakenly targets and destroys the insulin-producing β -cells within the pancreas. This autoimmune response compromises insulin production, resulting in

minimal to absent insulin levels. Consequently, glucose accumulates in the bloodstream rather than being transported into muscle and other tissues for cellular metabolism [6,7].

The administration of exogenous insulin is a critical component in the management of diabetes mellitus (specifically for Type-1), as it is necessary to prevent severe and potentially fatal complications resulting from sustained hyperglycemia. Since the discovery of insulin in 1921, there have been continuous advancements in the purity, potency, and half-life of insulin formulations. Initially, insulin was extracted from bovine and porcine pancreatic tissue; however, the development of recombinant DNA technology later enabled the large-scale production of human insulin [8]. Currently, insulin remains the cornerstone of pharmacological management for Type 1 diabetes mellitus (T1D). As a result, patients with (T1D) become entirely dependent on insulin therapy, which is typically administered parenterally via the subcutaneous route [9]. However, long-term insulin injections present several drawbacks, including pain at the injection site, allergic reactions, hyperinsulinemia, and insulin-induced lipodystrophy. These adverse effects significantly reduce patient compliance and adherence to treatment protocols, often resulting in poor glycemic control, increased clinical complications, psychological comorbidities, diminished overall well-being, and heightened mortality risk among individuals with diabetes [10 - 13]. In light of these challenges, oral insulin delivery has been explored as a potential alternative to eliminate injection-associated pain and to address psychological barriers, such as needle anxiety and infection risk. However, oral administration presents substantial obstacles, as insulin, being a protein, undergoes rapid enzymatic degradation in the acidic gastric environment and is further inactivated by proteolytic enzymes in the intestinal lumen [14, 18]. Moreover, insulin's high molecular weight and low lipophilicity contribute to its poor permeability across the intestinal epithelium. Consequently, the oral bioavailability of insulin remains extremely low and has been reported to be less than 2% [15-18]. Several pharmaceutical companies are actively developing oral insulin delivery systems,

with the majority of these efforts still in the clinical or pre-clinical stages of evaluation. At present, no orally administered insulin product has been approved for commercial use [19, 20]. The term *solid dispersion* refers to the dispersion of one or more active pharmaceutical ingredients within an inert carrier in the solid state. These systems are commonly prepared using methods such as melting (fusion), solvent evaporation, or a combination of both (fusion-solvent method). Within solid dispersions, the drug may exist in a molecularly dispersed form, as amorphous aggregates, or as crystalline particles [20-23]. The concept of utilizing solid dispersions for the oral delivery of insulin is based on the general advantages associated with these formulations. Solid dispersions are known to enhance the bioavailability of drugs, particularly those with poor water solubility, by improving their dissolution rate and absorption, thereby promoting a more effective therapeutic outcome [23, 24]. Solid dispersions have the potential to stabilize labile drugs by protecting them against various degradation processes, such as hydrolysis and oxidation [25]. Insulin, which exhibits poor solubility and stability in the gastric environment and possesses a molecular size that limits its passive diffusion from the intestinal lumen into the bloodstream, may substantially benefit from solid dispersion technology, especially for oral delivery applications [26]. Thus, it was hypothesized that encapsulating insulin in solid dispersions will enhance its solubility and bioavailability while offering protection against degradation in the harsh gastric environment. Solid dispersions can enhance the solubility and bioavailability of insulin while also offering protection against degradation in the harsh gastric environment. Commonly used polymers and copolymers in the preparation of solid dispersions include polyvinyl lactam-based polymers such as polyvinylpyrrolidone (PVP) and Soluplus®, cellulose derivatives like hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC), as well as polymethacrylates such as Eudragit [27-30]. In this study, hydroxypropyl methylcellulose (HPMC),

ethylcellulose, and Soluplus[®] were selected and combined in various ratios to formulate the carrier system for insulin.

Materials

Human Insulin (Humulin[®] 70/30) was purchased from Eli Lilly and Company, USA. Hydroxypropyl methylcellulose, Ethylcellulose, and Potassium dihydrogen phosphate were procured from Loba Chemie Pvt, Ltd, India. Soluplus[®] (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer) was a kind gift from BASF SE, Ludwigshafen, Germany. Ethanol was bought from JHD Chemicals, China. Fused Calcium chloride, Sodium hydroxide, and Sodium chloride were purchased from BDH, Poole, UK. Concentrated hydrochloric acid was procured from Guangdong Guanghua Sci-Tech Co., Ltd, China. Distilled water was obtained from an all-glass still.

Methods

Preparation of polymer matrix

Two polymeric matrices, designated as Matrix A and Matrix B, were formulated using different combinations of pharmaceutical grade polymers as shown in **Table 1**. Matrix A comprised three polymers: Soluplus[®], ethylcellulose (EC), and hydroxypropyl methylcellulose (HPMC). A 3 g quantity of each polymer was weighed using analytical weighing balance (Mxradly Lab., India) and dispersed in 100 mL of ethanol under continuous stirring using a magnetic stirrer (Searchtech Instrument 78HW-1, UK) at 1000 rpm for 12 h to ensure uniform mixing. Matrix B composed of two polymers: Soluplus[®] and HPMC, with a 5 g quantity of each polymer similarly dispersed in 100 mL of ethanol under constant agitation using a magnetic stirrer (Searchtech Instrument 78HW-1, UK) at 1000 rpm for 12 h. The resulting dispersions were transferred into clean, labelled containers and allowed to dry in a desiccator containing fused calcium chloride. The drying process was carried out at ambient temperature for seven (7) days.

After complete drying, the resulting thin polymeric films were collected and stored in air-tight containers for further use [31, 32].

Table 1: Composition of the different batches of the polymeric matrix

Ingredient	Soluplu®	HPMC	EC	Ethanol	Total weight	Dry weight
(g)	(g)	(g)	(g)	(g)	(g)	(g)
Matrix A	3.00	3.00	3.00	79.00	88.00	10.83
Matrix B	5.00	5.00	-	79.00	88.00	10.63

HPMC: hydroxypropyl methylcellulose; EC: Ethylcellulose

Preparation of insulin solid dispersions

Eight batches of solid dispersions, designated A1 to A4 and B1 to B4, were prepared using the solvent evaporation–milling technique as presented in **Table 2** [33, 34]. Each batch was formulated by dispersing 8 g quantity of the corresponding polymeric film (Matrix A or B) in 70 mL of ethanol with continuous stirring to ensure homogeneity. Insulin incorporation was carried out by adding specified volumes (3, 5, or 10 mL) of insulin to the first three batches of each polymeric film (A1–A3 and B1–B3). Batches A4 and B4 served as placebos containing no insulin. All formulations, both insulin-loaded and placebo were subjected to drying in a desiccator containing fused calcium chloride for 7 days at ambient temperature. After drying, the resulting polymeric films were milled using an electric blender to obtain granules of uniform particle size. The final solid dispersions were then stored in airtight containers pending further characterization and analysis [34].

Table 2: Composition of polymeric solid dispersions

Batch	Insulin (mL)	polymer matrix (g)	Ethanol (mL)
A1	3	8	70
A2	5	8	70
A3	10	8	70
A4	UNL	8	70
B1	3	8	70
B2	5	8	70
B3	10	8	70
B4	UNL	8	70

*UNL - Unloaded

Determination of yield

The weight measurement of the formulations was taken in triplicates before and after drying. And

The percentage yield was gotten using the formula as shown in equation 1:

$$\% \text{ yield} = \frac{\text{Weight of sample after evaporation (g)}}{\text{Initial weight of sample (g)}} \times 100 \quad - \quad - \quad - \quad -1$$

Micromeritic analysis

Determination of angle of repose

The angle of repose for the granules was determined using a fixed-height glass funnel mounted on a retort stand, positioned 3 cm above a flat surface. A 5 g quantity of granules was introduced into the funnel and allowed to flow freely through its orifice, forming a conical heap on the surface below. The height (h) of the resulting powder cone and the mean radius (r) of its base were measured. The angle of repose (θ) was then calculated using the following equation as shown in equation 2;

$$\tan \theta = h/r \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad -2$$

The experiment was conducted in triplicate for each formulation, and the average of three readings was recorded as the final angle of repose. To assess the effect of drop height on flow behaviour, the height of the funnel was also varied to 5 cm, 7 cm, and 10 cm, with each condition tested in triplicate [35, 36].

Determination of bulk and tapped densities

The bulk and tapped densities of the granules were determined using a dry, graduated measuring cylinder. A 5 g sample of granules (denoted as Wp) was carefully introduced into the cylinder, and the initial volume occupied (Vp) was recorded without any tapping. To determine the bulk density, the cylinder was tapped gently on a flat surface five times, and the corresponding volume was measured (Vb). For tapped density, the same sample was tapped 200 times on the table surface, and the final settled volume was recorded (Vt) [37, 38].

The densities were calculated using the following formulas in equations 3 and 4 respectively:

- Bulk Density (g/cm^3) = W_p/V_b - - - - - 3

- Tapped Density (g/cm^3) = W_p/V_t - - - - - 4

Determination of Carr's Compressibility Index and Hausner's ratio

Carr's compressible index (CI) and Hausner's ratio (HR) were calculated from the results obtained from bulk and tapped densities above using equations 5 and 6 respectively:

$$CI = 100(Td - Bd/Td) - - - - - 5$$

$$HR = \frac{\text{Tapped density}}{\text{Bulk density}} - - - - - 6$$

Where Td = Tapped density and Bd = Bulk density respectively.

Determination of encapsulation efficiency

A 0.5 g sample of each insulin-loaded solid dispersion batch was accurately weighed and immersed in 100 mL of ethanol. The mixture was left to stand for 48 hours to allow complete diffusion of insulin from the polymer matrix into the solvent. To facilitate the release of insulin,

the mixture was mechanically stirred to disrupt the solid dispersion and promote dissolution. Following this, 5 mL of each sample was withdrawn and placed in centrifuge tubes. The samples were centrifuged (Jenway 6505, East Norwalk, Connecticut, USA), for 5 min at 5000 rpm, and the resulting supernatant was carefully withdrawn using a syringe and transferred into a clean container. The concentration of insulin in the supernatant was determined using a UV-Visible spectrophotometer (Cary 60 by Agilent Technologies, Malaysia) at a wavelength of 272 nm. The encapsulation efficiency (EE %) and drug loading capacity (LC %) were calculated using the following formulas [39]:

$$\text{Encapsulation efficiency} = \frac{\text{Actual quantity of insulin encapsulated}}{\text{Total quantity of insulin in the formulation}} \times 100 \quad -7$$

$$\text{Loading capacity} = \frac{\text{Amount of insulin entrapped (g)}}{\text{Total weight of formulaion (g)}} \times 100 \quad -8$$

***In vitro* release**

The *in vitro* dissolution test was conducted using the paddle method, in accordance with the British Pharmacopoeia [20]. A volume of 900 mL of dissolution medium was used for each test: Simulated Gastric Fluid (SGF) with a pH of 1.2 and Simulated Intestinal Fluid (SIF) with a pH of 7.2. Both media were thermostatically maintained at 37 ± 0.5 °C throughout the experiment. The dissolution apparatus was assembled, and a known quantity of each formulation was enclosed in a dialysis membrane. This was then suspended using a retort stand and immersed into a glass beaker containing 900 mL of SGF. A magnetic stirrer, set at 50 rpm, was used to agitate the medium on an electronic heater/stirrer setup. At predetermined time intervals over a 2-hour period, 5 mL samples of the medium were withdrawn and immediately replaced with an equal volume of fresh SGF to maintain sink conditions. After the 2-hour SGF phase, the dialysis membrane was transferred into SIF (pH 7.2) for an additional 6-hour dissolution phase, with 5 mL aliquots withdrawn at specified time intervals. The same replacement technique was applied to maintain the volume.

Calibration curves for insulin were prepared using pure insulin in both SGF and SIF. These curves were used to quantify the amount of insulin released from each batch. The withdrawn samples were appropriately diluted and analyzed spectrophotometrically at 269 nm for SGF and 271 nm for SIF [40].

Fourier-transform infrared spectroscopy

In FTIR analysis, each sample was prepared by mixing 1 g of the solid dispersion with 0.5 g of potassium bromide (KBr) powder. To aid homogenization, 1 mL of Nujol was added to the mixture using a syringe, forming a consistent paste. The paste was then introduced into the sample mould and pressed into position for scanning. Spectral analysis was conducted over a wavelength range of 4000–6000 nm to obtain the characteristic FTIR spectra. Prior to scanning, the FTIR instrument (Buck scientific M530, Norwalk Connecticut, USA) was equipped with a deuterated triglycine sulphate (DTGS) detector and a potassium bromide beam splitter. The Gram A1 software was utilized to acquire and manipulate the spectra. All FTIR spectra were recorded and displayed as transmittance values, and peaks were analyzed to identify possible interactions between insulin and the excipients within the solid dispersion matrix [41].

DSC thermal analysis

The thermal behaviour of the polymers and insulin solid dispersions, including melting transitions, crystallization characteristics, and changes in heat capacity, was investigated using a differential scanning calorimeter (DSC) (TA Instruments, Waters Corporation, New Castle, USA). Approximately 4–5 mg of each sample (either polymer or formulation) was accurately weighed and sealed in an aluminium crucible, which was hermetically closed to prevent moisture or gas exchange. A similar empty aluminium crucible was used as the reference pan. Prior to sample analysis, the instrument was calibrated using indium as the standard. The system was purged with nitrogen gas at a constant flow rate of 20 mL/min to maintain an inert atmosphere during the scan. Thermal analysis was conducted by heating the samples from

30 °C to 400 °C at a rate of 10 °C/min, followed by a controlled cooling back to 30 °C at the same rate. Baseline correction was performed using the empty crucible, and all thermograms were adjusted accordingly to ensure accuracy [42].

Scanning electron microscopy

The morphological characteristics of the solid dispersions from each batch were examined using a Phenom ProX Scanning Electron Microscope (PhenomWorld, Eindhoven, Netherlands). For each formulation, a representative sample was mounted on a sample stub using double-sided adhesive carbon tape. The samples were then sputter-coated with a thin layer (approximately 5 nm) of gold using a Quorum Technologies model Q150R sputter coater to enhance conductivity. Following coating, the prepared samples were placed into the SEM chamber. Imaging was conducted at an accelerating voltage of 15 kV. Initial adjustments and focusing were performed using the NaVCaM system, and high-resolution micrographs were captured with the instrument's integrated digital camera. The images were subsequently processed and analyzed using Phenom Suite software to evaluate surface morphology and structural features of the granules [43, 44].

X-ray diffraction study

The crystalline orientations of the solid dispersions were evaluated using an X-ray diffractometer (XRD) (PANalytical, Almelo, The Netherlands). The XRD equipped with a nickel monochromator and set at 30mA and 40kV was used to analyze the samples including A2, A4, and B2 at ambient temperature in the range of 2θ (5 – 70°) at the rate of 0.5° per minute.

Stability study using pH measurements

The pH stability of the formulations was assessed over a three-month period. For each batch, 0.6 g of the sample was accurately weighed and dispersed in 100 mL of distilled water in a clean beaker. The pH of the resulting dispersion was measured using a pre-calibrated digital

pH meter (Hanna Instruments Inc, USA). The electrode was immersed in the dispersion, and readings were taken once the value stabilized. Each measurement was performed in triplicate to ensure reliability. This procedure was repeated monthly for a total duration of three months to monitor any pH changes indicative of potential degradation or instability of the formulations over time [45].

Animal studies

Animal care

Adult albino mice (*Mus musculus*) weighing between 17–22 g were obtained from the Animal House of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria with the ethical approval number: NAU/AREC/2021/0035. A total of 30 mice were used and randomly divided into six groups, each housed in standard laboratory cages under controlled environmental conditions. All animals had free access to standard rodent feed and clean drinking water ad libitum throughout the study period. The experimental protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University. All procedures involving animals were conducted in strict compliance with the European Economic Community (EEC) guidelines for the care and use of laboratory animals (Directive 2010/63/EU).

Antidiabetic activity studies

A total of thirty (30) adult albino mice were used for the anti-diabetic evaluation. The animals were randomly assigned into six groups (F1-F6), with five mice per group ($n = 5$) as shown in **Table 3**. Prior to the induction of diabetes, the mice were fasted for 18 hours with access to water ad libitum. Baseline fasting blood glucose levels were determined via tail vein puncture using a glucometer. Diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 120 mg/kg body weight. After 48 hours, hyperglycaemia was confirmed in the animals by measuring fasting blood glucose levels again with a glucometer.

Mice with blood glucose levels above 200 mg/dL were considered diabetic and selected for the study. Treatment was administered orally once daily. The diabetic groups received 200 mg/kg of the respective solid dispersion drug formulations or 1.5 IU of commercial insulin (Humulin® 70/30), depending on group allocation. Group F1 were treated with formulation A3 while F2-F5 were treated with formulations A1, B3, B1, and A4 respectively. Group F6 was treated pure insulin as the positive control. The blood glucose level was again taken at 1, 3, 5, 8, and 12 h post-treatment, blood was taken and blood glucose level was determined and the percentage glucose reduction was determined using the formular [46];

$$\% \text{ Glucose reduction} = \frac{A-B}{A} \times \frac{100}{1} \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad -8$$

where A= 0 h fasting blood glucose and B= Subsequent h Fasting blood glucose level (FBGL)

Table 3: Treatment groups

Groups	Drug formulations	Dose (mg)	Anhydrous insulin (mg)
F1	A3	4.20	1.400
F2	A1	3.80	0.273
F3	B3	4.00	1.300
F4	B1	3.80	0.320
F5	A4	3.80	0.000
F6	Pure Insulin		35.00

Haematology studies

Packed cell volume (PCV) evaluation

Packed Cell Volume (PCV) was determined using the microhematocrit (capillary tube) method. Blood samples were collected via retro-orbital (oculo-puncture) sampling into Eppendorf tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant.

Capillary tubes were then used to draw blood from the EDTA-treated samples. One end of each capillary tube was sealed with plasticine to prevent leakage. The sealed tubes were placed in a microhematocrit centrifuge with the sealed ends positioned outward, firmly against the rubber gasket to ensure proper balancing. Centrifugation was carried out at 1200 rpm for 10 minutes. After centrifugation, the capillary tubes were transferred to a haematocrit reader to determine the proportion of packed red blood cells. The PCV values were recorded and expressed as a percentage of the total blood volume [47].

Determination of red blood cell (RBC) Count

The red blood cell count was determined using the haemocytometer method. Blood samples were collected via oculo-puncture and transferred into EDTA-containing tubes to prevent coagulation. Using an RBC pipette, the blood sample was drawn up to the 0.5 mark, after which Hayem's solution (RBC diluting fluid) was aspirated into the pipette up to the 101 mark. The contents were thoroughly mixed by shaking the pipette gently. A clean cover glass was placed over the counting chamber of the haemocytometer. The tip of the RBC pipette was then brought into contact with the edge of the cover glass, allowing the chamber to fill by capillary action without air bubbles. The setup was left undisturbed for approximately 2 minutes to allow the cells to settle. The chamber was placed under a light microscope and initially examined using the low-power objective (10x) to locate the grid. RBCs were then counted in the appropriate squares using the high-power objective (40x), and the total RBC count was calculated accordingly [48].

Estimation of the haemoglobin

The hemoglobin concentration was determined using Sahli's (acid hematin) method. A Sahli hemoglobinometer was used for the analysis. The graduated dilution tube was filled with fresh 0.1 N hydrochloric acid up to the reference mark. Using an RBC pipette, 20 mm³ (20 µL) of blood was drawn and introduced into the acid solution in the dilution tube. The contents were mixed thoroughly by drawing the mixture up and down in the pipette several times, ensuring uniform dispersion. The tube was left undisturbed for 5 minutes to allow complete conversion of hemoglobin to acid hematin. Following this, distilled water was added dropwise, while continuously mixing with a glass stirring rod after each drop, until the solution's color matched the permanent comparator standard in the hemoglobinometer. The hemoglobin concentration was then read at the upper meniscus of the fluid column in the dilution tube and recorded in grams per deciliter (g/dL) [49].

Determination of alanine transaminase (ALT) and aspartate aminotransferase (AST)

The ALT and AST activity was measured using a commercially supplied enzymatic kit based on the Reitman-Frankel or IFCC method. The reagents supplied in the kit—Reagent 1 (R1) and Reagent 2 (R2) were mixed in a 5:1 ratio to prepare the working reagent. Clean test tubes were labelled as “Sample” and “Blank”. Into each tube, 1.0 mL of the working reagent was dispensed. The tubes were pre-incubated for 5 minutes at 37 °C in a water bath or dry heat block. To each sample tube, 100 µL of serum sample was added. To the blank, 100 µL of distilled water was added instead of serum. The reaction mixtures were briefly mixed and then immediately transferred to a spectrophotometer cuvette. The absorbance was read at 340 nm at exactly 1 minute and again at 3 minutes. The change in absorbance (ΔA/min) was used to calculate ALT and AST activity using the kit's provided formula or factor as follows:

$$\text{ALT/AST (U/L)} = \frac{\Delta A}{\text{min}} \times \frac{1768}{1} - \quad - \quad - \quad - \quad - \quad - \quad -9$$

The activity of alkaline phosphatase (ALP) was determined using a commercially supplied colorimetric kit based on the p-nitrophenyl phosphate (pNPP) method. Fresh test tubes were

labeled as “Standard,” “Sample,” and “Blank.” Into each tube, 0.5 mL of ALP substrate reagent was dispensed. The tubes were then equilibrated in a water bath at 37 °C for 3 minutes. To the standard tube, 50 µL of standard solution (provided in the kit) was added. To the sample tube, 50 µL of the test serum was added and finally 50 µL of deionized water was added to the blank. The contents were mixed gently and the tubes were incubated at 37 °C for 10 minutes. After incubation, 2.5 mL of ALP color developer reagent (from the kit) was added to each tube and mixed thoroughly. The absorbance was measured at 600 nm using a spectrophotometer. The ALP concentration was calculated using the formula provided in the kit, typically [50]:

$$\frac{\text{Abs.of sample}}{\text{Abs.of standard}} \times \text{Concentration of standard} - \quad - \quad - \quad - \quad -10$$

Evaluation of blood urea nitrogen (BUN)

The concentration of blood urea nitrogen (BUN) was determined using a colorimetric enzymatic method with a commercial urea/BUN assay kit. The BUN enzyme reagent was reconstituted according to the manufacturer’s instructions. Fresh test tubes were labeled as “Blank,” “Sample,” and “Standard.” A 1.5 mL volume of the reconstituted BUN enzyme reagent was pipetted into each tube and allowed to equilibrate at room temperature for 5 minutes. To the Blank, 10 µL of deionized water was added. To the Sample tubes, 10 µL of each test sample was added and finally to the Standard tube, 10 µL of the standard reagent provided in the kit was added. All tubes were mixed thoroughly and incubated for 5 minutes at 37 °C. After incubation, the absorbance of each tube was read at 630 nm using the blank to zero the spectrophotometer. The concentration of the urea nitrogen was calculated using the formular [51]:

$$\frac{\text{Abs.of unknown (test)}}{\text{Abs.of the standard}} \times \text{Concentration of standard} \quad - \quad - \quad - \quad - \quad 11$$

Determination of creatinine

Serum creatinine concentration was determined using the alkaline picrate (Jaffe) method via a colorimetric assay kit. Equal volumes of the creatinine picric acid reagent and creatinine buffer

reagent supplied in the kit were thoroughly mixed to prepare the working reagent. Clean test tubes were labeled as “Blank,” “Standard,” and “Test” (or by sample group names). A 3.0 mL volume of the working reagent was pipetted into each tube. To the blank, 100 µL of distilled water was added. To the standard, 100 µL of the provided standard solution was added. Finally, to each sample tube, 100 µL of the respective serum sample was added. All tubes were mixed gently and incubated in a water bath at 37 °C for 15 minutes. After incubation, the tubes were cooled to room temperature, and the absorbance was measured at 520 nm, using the blank to zero the spectrophotometer. The creatinine value was calculated using the formular [52];

$$\frac{\text{Abs.of unknown (test)}}{\text{Abs.of standard}} \times \text{Concentration of standard} - \quad - \quad - \quad - \quad - \quad 12$$

Statistical analysis

Data are given as Mean ± standard deviation (SD). Difference between groups were evaluated using students’ T-test. Significant differences were defined by $p < 0.05$.

Results and discussion

Polymer yield

The initially white polymers formed a clear, colorless solution. Following solvent evaporation, the dried residues exhibited distinct appearances: matrix A yielded a golden film, while matrix B remained white as presented in **Table 4**. This visual difference may suggest varying interactions between the polymer components and ethanol or differential recrystallization behavior during drying [28, 29]. The unloaded (blank) samples displayed the highest weights among the batches. A progressive decrease in weight was observed with increasing insulin incorporation. This trend implies that the added insulin displaced a portion of the polymer matrix, thereby reducing the overall mass of the solid dispersion, while maintaining the same total formulation volume. Among all the batches, Batch A3 exhibited the highest percentage yield, recorded at 12.22%. Interestingly, formulations containing two polymers demonstrated higher weights compared to those combining three polymers. This may be attributed to

differences in polymer compatibility, miscibility, or packing density, which can influence the final mass and structural integrity of the dried matrix [30].

Table 4: Compositions and percentage yield of polymeric solid dispersion

Ingredient (g)	A1	A2	A3	A4	B1	B2	B3	B4
Matrix A	8.00	8.00	8.00	8.00	-	-	-	-
Matrix B	-	-	-	-	8.00	8.00	8.00	8.00
Ethanol	55.00	55.00	55.00	55.00	55.00	55.00	55.00	55.00
Insulin	0.04	0.02	0.01	-	0.04	0.02	0.01	-
Total weight	63.04	63.03	63.01	63.00	63.04	63.03	63.01	63.00
Dry weight	4.99	5.63	7.70	7.63	5.40	6.00	6.56	7.28
% yield	7.92	8.93	12.22	12.11	8.57	9.52	10.41	11.56

Micromeritic parameters of the granules

The flow properties of the granules are influenced by the degree of inter particulate friction, which determines how easily the particles move relative to one another [31]. In this study, the granules exhibited good flowability, as indicated by an angle of repose of less than ($< 25^\circ$), which is typically characteristic of materials with excellent flow as presented in **Table 5** [32]. Bulk and tapped density values ranged from $0.50 \pm 0.01 \text{ g/cm}^3$ to $0.70 \pm 0.01 \text{ g/cm}^3$, reflecting the packing ability of the granules and their suitability for encapsulation or compression [33]. The compressibility index and Hausner's ratio, which are indirect indicators of powder cohesiveness and flow behaviour, are in agreement with the obtained results. These parameters reflect interparticulate interactions, where a closer alignment between bulk and tapped densities

results in a lower compressibility index and improved flow characteristics. All samples demonstrated Hausner's ratios below 1.25 and compressibility indices ranging between $2.13 \pm 1.01\%$ and $6.61 \pm 0.90\%$, indicating excellent flow properties [34]. Among the batches, sample A2 showed the most favorable flow characteristics. These results suggest that the granules were well-suited for further downstream processing, such as tableting or capsule filling.

Table 5: Micromeritic properties of polymeric solid dispersions (Mean $\pm$ S.D, n=3)

Formulation	Angle of repose	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Compressi bility index (%)	Hausner's ratio
A1	21.34 $\pm$ 0.98	0.52 $\pm$ 0.01	0.55 $\pm$ 0.01	6.61 $\pm$ 0.90	1.07 $\pm$ 0.01
A2	23.64 $\pm$ 1.10	0.57 $\pm$ 0.01	0.58 $\pm$ 0.01	2.28 $\pm$ 1.01	1.02 $\pm$ 0.01
A3	23.16 $\pm$ 1.40	0.61 $\pm$ 0.01	0.62 $\pm$ 0.01	2.13 $\pm$ 0.90	1.02 $\pm$ 0.01
A4	21.11 $\pm$ 1.79	0.63 $\pm$ 0.01	0.65 $\pm$ 0.01	3.05 $\pm$ 1.49	1.03 $\pm$ 0.02
B1	18.73 $\pm$ 1.09	0.64 $\pm$ 0.01	0.67 $\pm$ 0.01	3.97 $\pm$ 0.47	1.04 $\pm$ 0.01
B2	18.06 $\pm$ 0.98	0.56 $\pm$ 0.01	0.59 $\pm$ 0.01	4.51 $\pm$ 0.53	1.05 $\pm$ 0.01
B3	16.84 $\pm$ 0.79	0.56 $\pm$ 0.02	0.59 $\pm$ 0.01	5.61 $\pm$ 2.53	1.06 $\pm$ 0.03
B4	11.84 $\pm$ 0.70	0.7 $\pm$ 0.01	0.73 $\pm$ 0.01	2.77 $\pm$ 0.55	1.03 $\pm$ 0.00

Encapsulation efficiency and loading capacity

The encapsulation efficiency of the insulin-loaded solid dispersions ranged from 54% to 78%, while the loading capacity varied between 16% and 48% as shown in **Table 6**. These values

demonstrated the polymers' capacity to accommodate insulin within their matrix, likely due to favorable physicochemical interactions during the formulation process. Among the batches, A1 recorded the highest EE at 78.69% and LC at 48.17%, suggesting a strong polymer-drug interaction and optimal encapsulation conditions. A2 and A3 had EE values of 56.91% and 62.89%, with corresponding LC values of 27.63% and 18.45%, respectively. Similarly, batches B1, B2, and B3 exhibited EE values of 61.03%, 54.21%, and 62.56%, and LC values of 41.75%, 22.37%, and 16.54%, respectively. The high LC values, particularly in batches A1 and B1, indicate that the polymer matrices were able to incorporate sufficient amounts of insulin, potentially enabling significant therapeutic efficacy in lowering blood glucose levels. These findings suggest that the developed solid dispersions are promising carriers for insulin delivery in the management of diabetes mellitus [35, 37].

Table 6: Encapsulation efficiency and loading capacity of insulin loaded polymeric solid dispersions

Formulation	Concentration (iu/ml)	Encapsulation efficiency (%)	Loading capacity (%)
A1	2.87	78.69	48.17
A2	0.64	56.91	27.63
A3	0.57	62.89	18.45
B1	0.46	61.03	41.75
B2	0.11	54.21	22.37
B3	0.29	62.56	16.54

***In vitro* dissolution**

The *in vitro* dissolution profiles of formulations A1–A3 and B1–B3 are presented in Figures 1 and 2, respectively. The release studies were conducted using a two-stage dissolution model: Simulated Gastric Fluid (SGF, pH 1.2) for the initial 2 hours, followed by Simulated Intestinal Fluid (SIF, pH 7.2) for the remaining 6 hours, giving a total duration of 480 minutes. This model mimics the physiological transition of oral dosage forms through the gastrointestinal tract [37].

Among the three-polymer blend formulations (Figure 1), batch A2 (5 mL insulin) exhibited the highest release, with 80% insulin released within 80 minutes and 18% released up to 360 minutes. Batch A1 (10 mL insulin) exhibited a biphasic release pattern, achieving 53% release at 100 minutes and 38% at 180 minutes. In contrast, batch A3 (3 mL insulin) demonstrated a more limited release, with 12% at 90 minutes and only 7% at 480 minutes.

For the two-polymer blends (Figure 2), batch B1 (10 mL insulin) showed the highest release with 58% at 100 minutes and 42% at 480 minutes. Batch B2 (5 mL insulin) and B3 (3 mL insulin) showed moderate release, with B2 achieving 18% at 90 minutes and 30% by 480 minutes, while B3 reached 20% at 100 minutes and 29% at 180 minutes.

The observed release profiles correlated with the pH characteristics of the formulations. Batch A formulations exhibited a more acidic pH of 3.38 ± 0.05 , which favored a higher insulin release in the acidic SGF. Conversely, batch B formulations had a pH closer to neutrality (6.67 ± 0.45), promoting greater release in the alkaline SIF environment. This suggests that the dissolution behavior was influenced by pH-dependent solubility and polymer-drug interactions [29, 47].

Furthermore, results obtained showed that the drug was released from the polymer matrix at variable time intervals, with peak release occurring between 90 and 100 minutes across all

batches. This period may represent the onset of maximum therapeutic activity. Importantly, the sustained release behavior observed during the intestinal phase suggests that the formulations exhibit characteristics of modified-release systems. Such systems can prolong drug release, enhance mucosal absorption, and ultimately improve the oral bioavailability of insulin [35, 36]. Additionally, the lack of degradation in the gastric phase indicates good stability of insulin in the simulated acidic medium, an essential factor for successful oral peptide delivery.

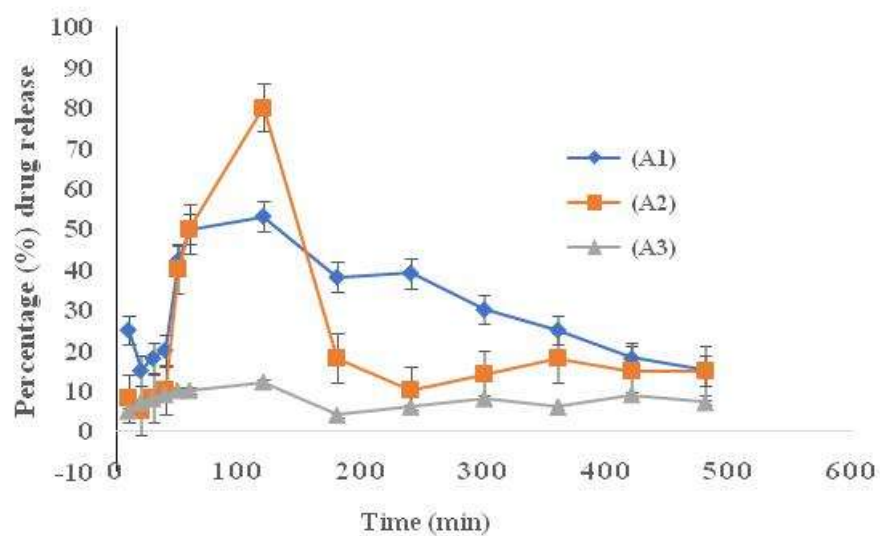


Figure 1: *In vitro* release curves of batches A1, A2 and A3.

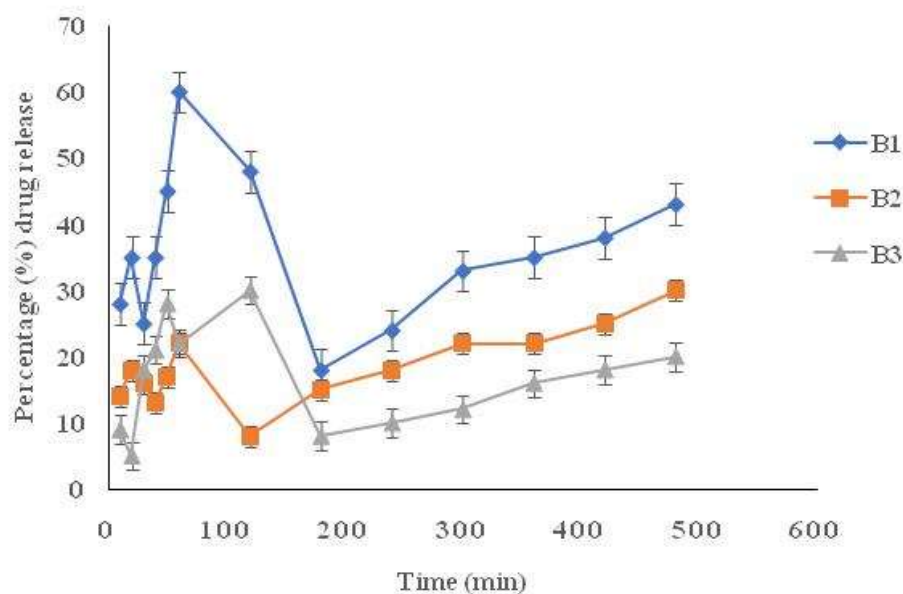


Figure 2: *In vitro* release curve of batches B1, B2 and B3.

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of pure insulin exhibited characteristic absorption peaks at 1607.59 cm^{-1} (C=C stretching), 1825.43 cm^{-1} (C=O stretching), 2528.41 cm^{-1} (S-H stretching of alkyl mercaptan), and 3394.54 cm^{-1} (O-H stretching of alcohols), confirming the presence of key functional groups associated with the peptide structure of insulin as shown in **Fig. 3a**. The FT-IR spectrum of formulation B1 (**Fig. 3b**) revealed bands at 1628.41 cm^{-1} (C=C stretching), 1785.98 cm^{-1} (C=O stretching), 2948.56 cm^{-1} (C-H stretching), 3244.09 cm^{-1} (N-H stretching), and 3439.43 cm^{-1} (O-H stretching). Similarly, A1 (**Fig. 3c**) displayed absorption peaks at 1624.67 cm^{-1} (C=C stretching), 1896.47 cm^{-1} (C-N stretching), 2795.96 cm^{-1} (C-H stretching), 3201.68 cm^{-1} (N-H₂ stretching), and 3403.45 cm^{-1} (O-H stretching). The unloaded batch A4 (**Fig. 3d**) presented peaks at 1622.79 cm^{-1} , 1879.71 cm^{-1} , and 3215.65 cm^{-1} , corresponding to C=C stretching, N-H bending, and O-H stretching, respectively. The presence of functional groups such as -OH, -C=O, -SH, -NH₂, and -C=C in both the pure insulin and the loaded formulations, with minimal shifts or changes in peak positions, indicates

the absence of significant chemical interaction between insulin and the polymer matrix. This suggests that the structural integrity of insulin was preserved during formulation and that no covalent bonding or chemical degradation occurred during the dispersion process. These findings confirm the chemical compatibility between insulin and the polymer carriers used in the solid dispersion formulations. The minor peak shifts observed are most likely due to physical interactions such as hydrogen bonding or entrapment within the polymeric matrix rather than chemical modifications [30]. This compatibility is essential for ensuring the stability and bioactivity of insulin throughout formulation and delivery.

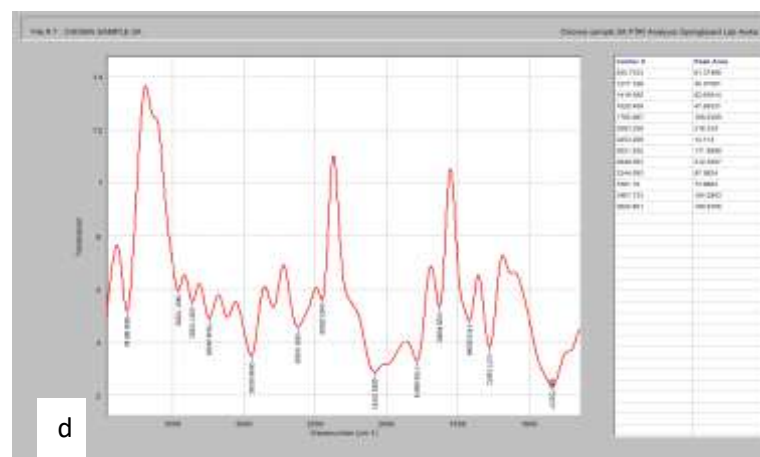
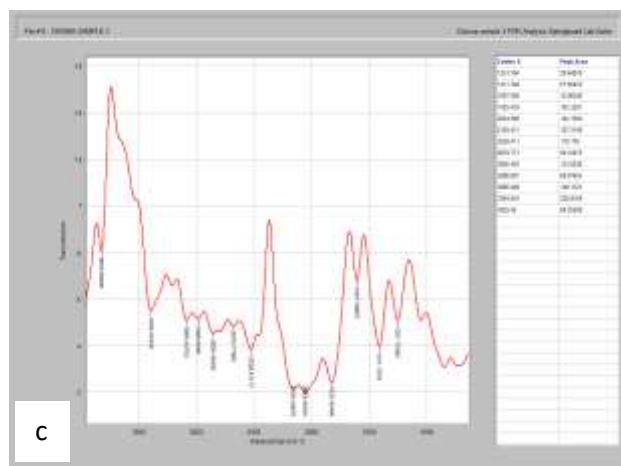
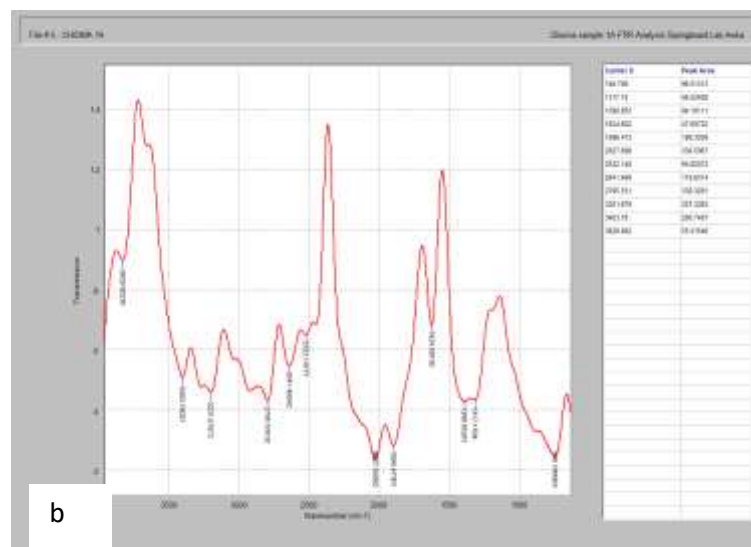
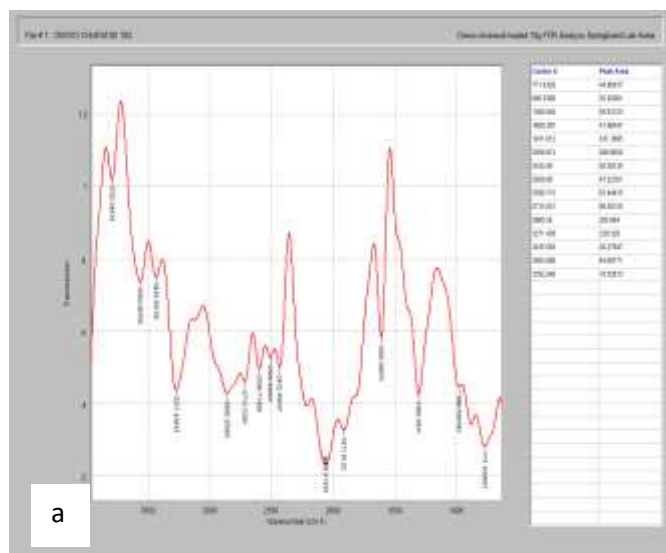


Figure 3: FTIR spectra of (a) Insulin (b) B1 (c) A1 (d) A4

Thermal analysis

The thermal behavior of the formulations was evaluated using Differential Scanning Calorimetry (DSC), and the thermograms are shown in Figures (4a-c). The DSC thermogram of the unloaded formulation (Batch A4) (**Fig. 4a**) exhibited a sharp endothermic peak at 91.69 °C (Figure 4a), indicating a high degree of crystallinity within the polymer matrix. In contrast, the thermograms of loaded formulations A3 and B3 showed endothermic peaks at 92.33 °C and 100.07 °C, respectively (**Figures 4b and 4c**), with broader and less intense peaks compared to A4. The shift in melting peaks and the reduction in sharpness for batches A3 and B3 suggest a disruption of the crystalline structure, indicative of a transition toward an amorphous state upon insulin loading. This transition is likely a result of molecular dispersion of the drug within the polymer matrix, which reduces the ordered arrangement of the polymer chains [38]. The amorphization of a drug is known to enhance its dissolution rate due to higher internal energy and improved wettability [29]. Batch A3 presented a moderately sharp peak, suggesting partial crystallinity and a semi-amorphous nature. In contrast, Batch B3 presented a much broader peak, indicative of a higher amorphous content. These observations imply that the two-polymer blend in Batch B3 disrupted the crystalline lattice more effectively than the three-polymer blend in Batch A3. The more amorphous nature of Batch B3 suggests a higher potential for improved dissolution and enhanced drug release, which is in agreement with the dissolution data presented earlier. Overall, these findings confirm that the polymer blend composition significantly affects the thermal and physical properties of the insulin-loaded formulations, and that increased amorphous character may enhance the bioavailability of poorly soluble drugs like insulin.

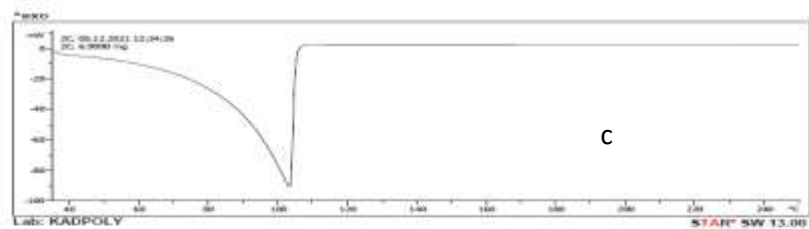
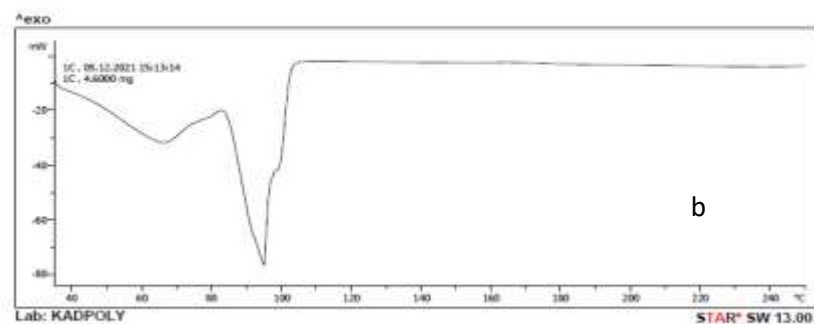
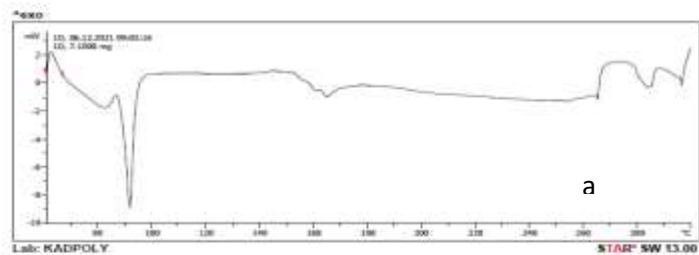


Figure 4: DSC thermograms of (a) A4, (b) A3 (c) B3

Scanning electron microscope (SEM)

SEM analysis showed notable differences between unloaded and insulin-loaded batches as shown in Figure 5a-c. The unloaded batches exhibited a tightly packed structure with minimal surface porosity, indicating a dense polymer matrix. In contrast, the loaded batches displayed more irregular surfaces with visible voids and rough undulations across the matrix. These morphological features suggest that insulin incorporation introduced structural modifications, likely forming channels or pores that facilitate drug entrapment within the polymer network. The presence of these surface discontinuities and open regions in the loaded samples supports the hypothesis that the polymer matrix was successfully modified to accommodate insulin. Such morphological changes are critical as they influence not only drug loading but also the rate and mechanism of drug release [39]. The observed porous structures in the loaded formulations may enhance dissolution by increasing surface area and promoting faster hydration and erosion of the matrix. Overall, the SEM images confirm that drug incorporation affects the microstructure of the solid dispersions, providing further evidence of successful formulation design aimed at improving drug release and bioavailability.

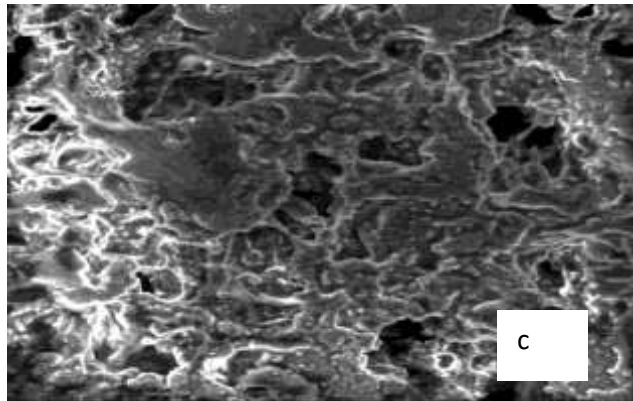
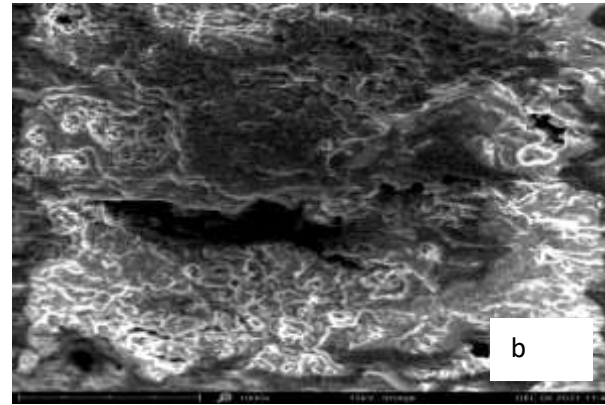
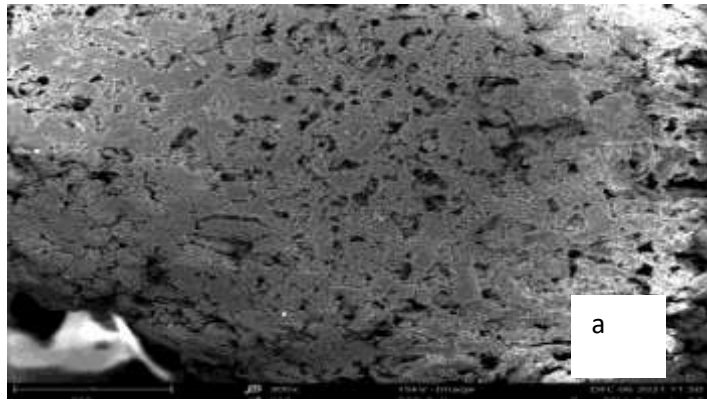


Figure 5: (a) A4, (b) A3, (c) B3

X-ray diffraction

The diffraction patterns of samples A2, B2, and the unloaded reference sample A4 revealed distinct peaks on the 2θ axis, indicating varying degrees of crystallinity and amorphous content as shown in **Figure 6**. A2 exhibited a prominent peak at 23° , while B2 showed a peak at 20° . These values are comparable to the peak observed at 22° in the unloaded sample A4, which served as the reference. The similarity in peak positions across the loaded and unloaded samples suggests that no significant chemical interaction or incompatibility occurred between the drug and the polymer carriers [40]. This affirms that the drug was incorporated into the polymer matrix without altering the intrinsic crystalline structure of the excipients.

However, differences in peak intensity and sharpness were observed. The loaded samples (A2 and B2) demonstrated higher intensities than the unloaded A4, which can be attributed to the presence of crystalline insulin. Notably, B2 showed a broader peak compared to A2, indicating a greater degree of amorphization [41, 42]. The broader diffraction in B2 suggests that the two-polymer system was more effective in disrupting crystalline order and promoting the formation of an amorphous matrix, which is generally associated with enhanced drug solubility and bioavailability. Conversely, A4 displayed a sharp and intense peak at 22° , signifying a highly crystalline structure typical of unloaded polymers. The reduction in peak sharpness and the increase in amorphous content in the loaded formulations implied that drug incorporation reduced the crystallinity of the polymer matrix. This transformation is beneficial in enhancing the dissolution behaviour and improving the oral bioavailability of poorly water-soluble drugs such as insulin.

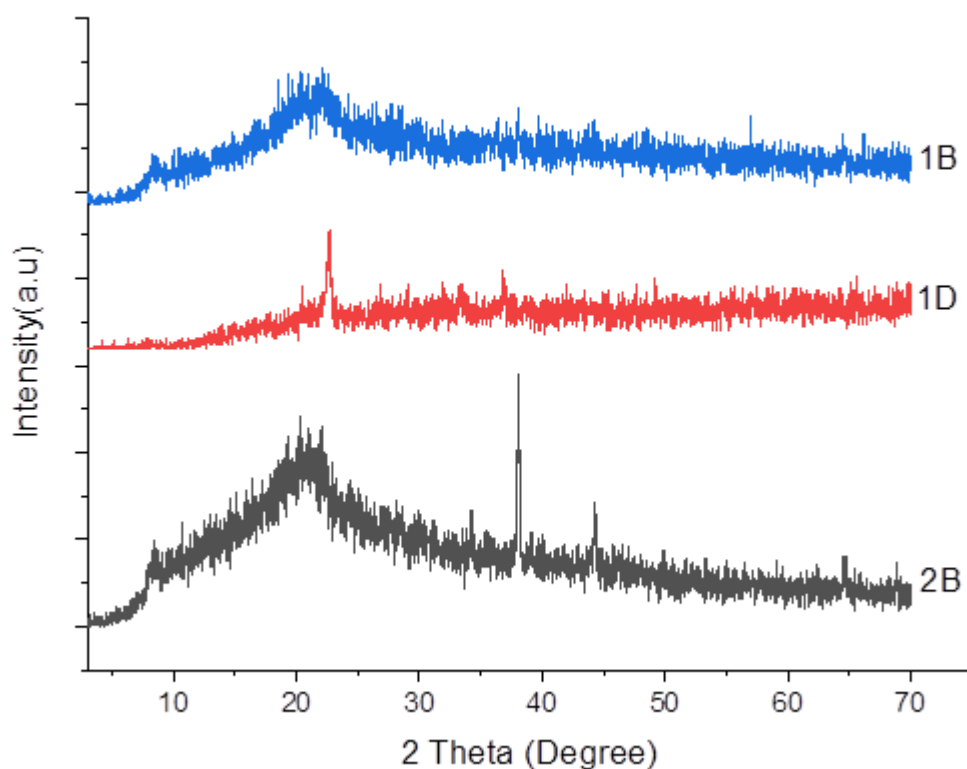


Figure 6: XRD superimposed graph with A2 coded as 1B, A4 coded as 1D, and B2 coded as 2B

Anti-diabetic activity studies

The percentage glucose reduction in each group is shown in **Figure 7**. The percentage reductions in blood glucose levels following oral administration of insulin-loaded polymeric solid dispersions were 62.08%, 65.94%, and 79.26% for Groups F1, F2, and F3, respectively. Among the treatment groups, Group F3, which received the B3 solid dispersion, demonstrated the most pronounced hypoglycaemic effect, achieving a 79.26% reduction in blood glucose levels over the 12-hour observation period. This result indicates superior pharmacodynamic performance and a prolonged duration of action. Furthermore, the onset of hypoglycaemic effect in this group was both rapid and sustained throughout the study period without noticeable attenuation. These findings highlight the potential of combining Soluplus® and hydroxypropyl methylcellulose (HPMC) as an effective polymer matrix blend for enhancing the therapeutic

efficacy of orally administered insulin. The sustained hypoglycaemic response is attributable to the properties of the polymers used, which include small particle size, high surface area, strong adaptability, and controlled-release behaviour. These characteristics are known to improve the stability of insulin in the gastrointestinal tract, protect it from enzymatic degradation, and enhance mucosal absorption, thereby increasing its oral bioavailability [43]. Furthermore, the polymeric solid dispersions effectively encapsulated insulin, thereby protecting it from degradation in the gastrointestinal tract. This protective effect was confirmed by the significant anti-diabetic activity exhibited by all insulin-loaded formulations within the 12-hour testing period. This protective mechanism prevented premature degradation and facilitated sustained drug release at the site of absorption, consistent with previous reports on oral peptide drug delivery using nanocarriers [44]. In comparison, Groups F1 and F2 exhibited substantial reductions in blood glucose levels, achieving 62.08% and 65.94% reductions, respectively. These findings underscore the effectiveness of the solid dispersions formulated with three-polymer blends in enhancing oral insulin delivery. Although their hypoglycaemic effects were slightly lower than that observed in Group F3, both groups still demonstrated sustained insulin release over the observation period. Moreover, these formulations provided protective effects against degradation in the harsh gastrointestinal environment, as evidenced by their maintained pharmacological activity. In contrast, Group F6, which received insulin via subcutaneous injection, demonstrated the lowest percentage reduction in blood glucose (1.13%) within the same 12-hour period. This unexpectedly poor performance may be explained by various physiological factors that affect subcutaneous absorption, including local blood flow, injection volume and depth, tissue diffusion capacity, and body movement during the absorption phase [45]. These variables can significantly influence the bioavailability and pharmacokinetics of subcutaneously administered peptides. Group F5 showed a modest glucose reduction (9.84%), despite receiving a formulation without active insulin. This

reduction is likely attributable to residual β -cell function. Alloxan, the diabetogenic agent used to induce hyperglycaemia in the study, selectively impairs pancreatic β -cells but may not completely destroy them at sub-lethal doses [46]. Therefore, endogenous insulin production might have been partially preserved, contributing to the observed glucose-lowering effect. Taken together, the findings demonstrate that oral delivery of insulin via polymeric solid dispersions presents a promising alternative to conventional subcutaneous administration. The formulation not only protected insulin from enzymatic degradation but also provided sustained glycaemic control, indicating its potential utility in the management of diabetes mellitus as shown in **Figure 7**.

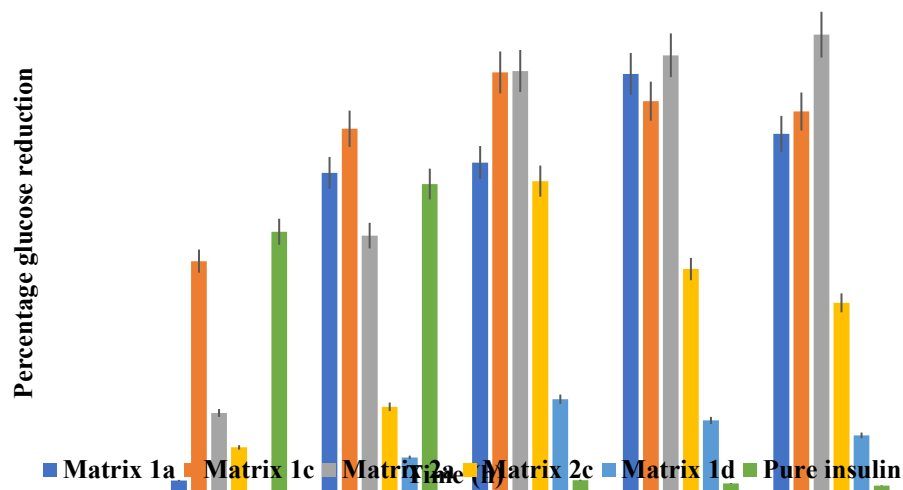


Figure 7: Percentage (%) glucose reduction with time (h).

Hematological studies

Oral administration of the insulin formulations in groups F1, F2, F3, and F4 produced no statistically significant changes ($p > 0.05$) in haematological parameters, including packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell (RBC) count as shown in **Figure 8**. This suggests that these formulations do not exert haematological toxicity and are

likely to be safe for therapeutic use. Comparable findings were observed in group F6 (commercial insulin), which also did not induce significant alterations in haematological parameters, serving effectively as a positive control. Conversely, group F5 (formulation without insulin) exhibited statistically significant changes ($p < 0.05$) in the same haematological parameters. The absence of insulin in this group likely led to sustained hyperglycaemia, which can induce oxidative stress and subsequent haematological toxicity. This outcome supports the critical role of insulin in maintaining glucose homeostasis and preventing blood-related complications associated with chronic hyperglycaemia [48]. These results indicate that polymer-based oral insulin formulations containing the active drug demonstrate a safety profile comparable to commercial insulin, while the absence of insulin can lead to adverse haematological effects due to uncontrolled blood glucose levels.

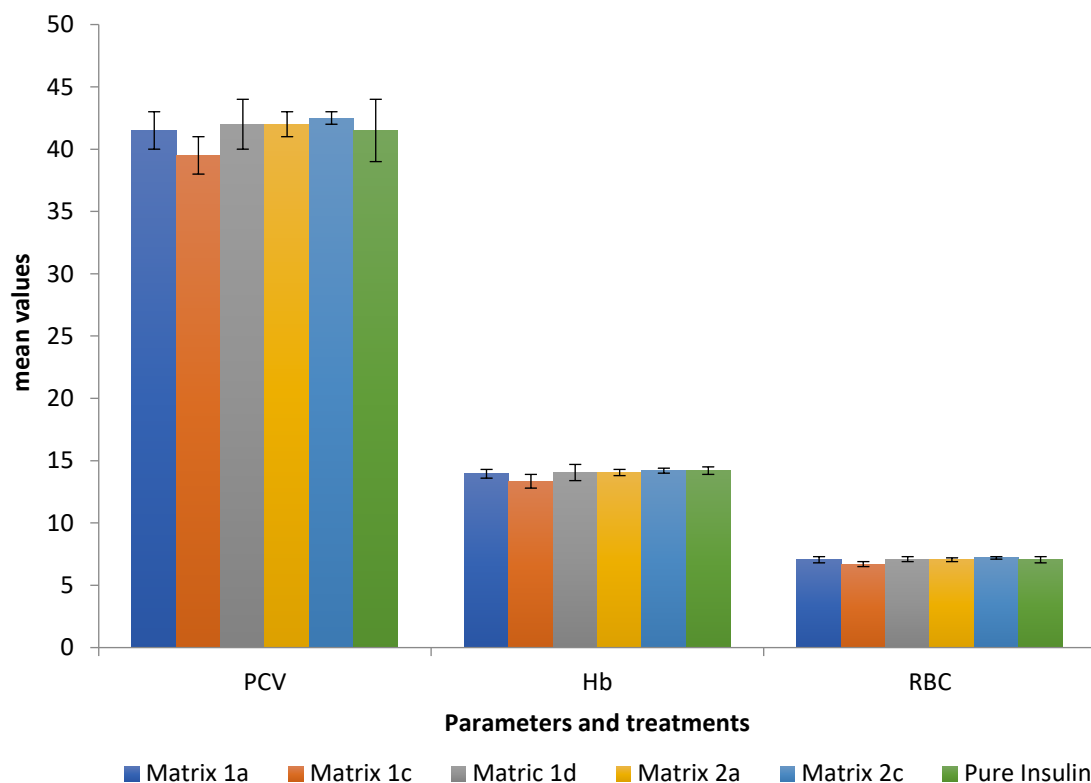


Fig 8: A bar chart representation of mean haematological values against treatments.

Liver and kidney marker (biochemical parameters) studies

Oral administration of the insulin formulations in groups F1 and F2 produced no statistically significant changes ($P > 0.05$) in the evaluated biochemical parameters, including liver function tests (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST)) and kidney function tests (urea and creatinine clearance) as shown in **Figure 9**. These findings suggest that these polymer-based insulin formulations do not induce hepatotoxicity or nephrotoxicity and may therefore be considered biochemically safe for therapeutic use. In contrast, groups F3 and F4 showed statistically significant elevations ($p < 0.05$) in ALT and AST levels—key markers of hepatocellular injury. This indicates that these particular formulations may exert adverse effects on the liver and should be administered cautiously to minimize potential hepatotoxic risk. Group F5, which contained no active ingredient (insulin), exhibited significant changes ($p < 0.05$) across all evaluated biochemical parameters. This is likely due to persistent hyperglycaemia and associated glucose toxicity, as uncontrolled high blood glucose is known to induce oxidative stress and compromise liver and kidney function [48-51]. As expected, group F6, the commercial insulin formulation (Humulin 70/30), did not show significant alterations ($p > 0.05$) in any of the biochemical parameters, reinforcing its established safety profile as a positive control. These results collectively demonstrate that polymer-based oral insulin formulations containing insulin (especially F1 and F2) appear biochemically safe, whereas formulations either lacking insulin or certain polymer–insulin combinations (F3 and F4) may pose hepatotoxic risks.

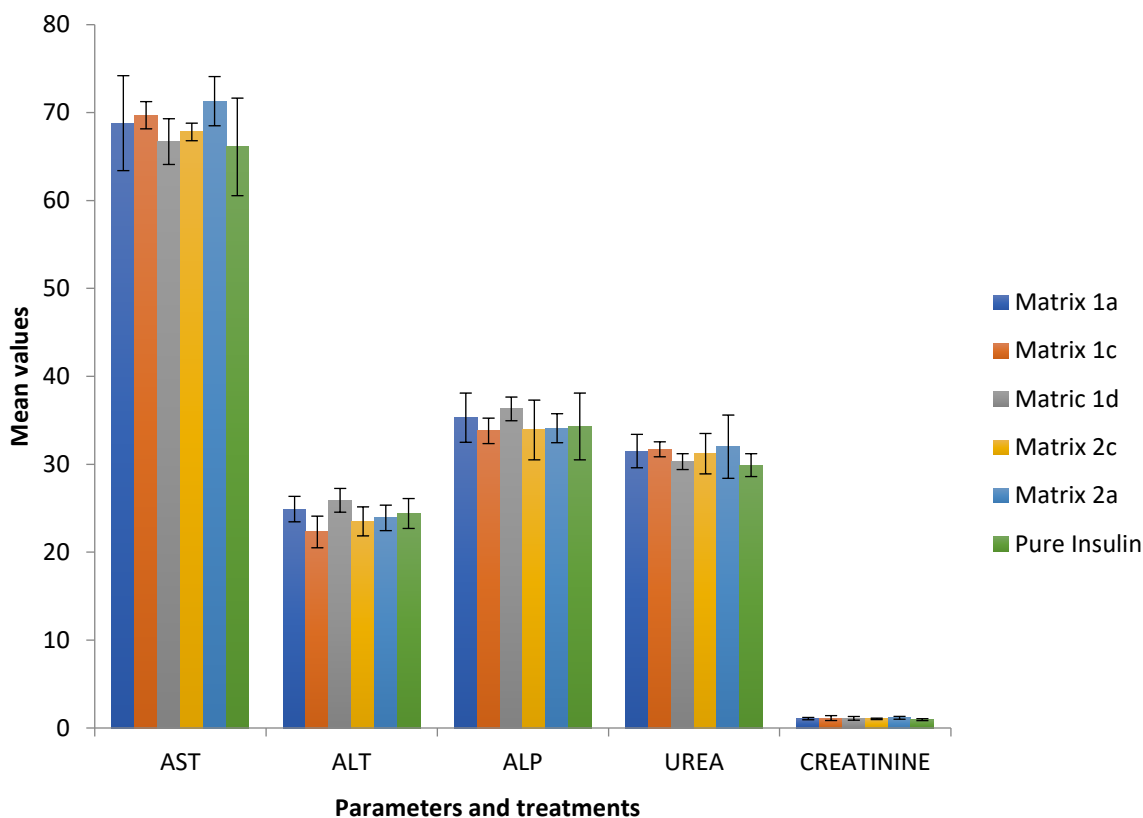


Fig 9: A bar chart representation of mean biochemical parameters against treatments

Stability studies

The results of the stability study, presented in **Table 7**, showed that after three months of storage, there was no statistically significant change in the pH of the formulations. Native insulin typically has a pH range of approximately 7.0–7.8 [49]. The batch B formulations, which contained Soluplus[®] and hydroxypropyl methylcellulose (HPMC), exhibited pH values closer to this physiological range. In contrast, batch A formulations demonstrated more acidic pH values, likely attributable to the inclusion of an additional polymer, ethyl cellulose. The presence of ethyl cellulose appears to shift the formulation pH towards acidity, which could be advantageous by enhancing compatibility with the acidic gastric environment and potentially improving insulin stability and absorption upon oral administration [52]. This formulation strategy may thus contribute to protecting insulin against rapid degradation in the stomach, which is a key barrier to effective oral delivery. Furthermore, the observation that the

formulations maintained a relatively stable pH over three months suggests chemical stability at room temperature. This indicates that these solid dispersions, may circumvent the absolute dependence on cold chain storage typically required for parenteral insulin products [53, 54]. The polymeric matrix likely plays a protective role, shielding insulin from environmental degradation and preserving its structural integrity. These findings support the potential of polymer-based solid dispersion formulations to offer both enhanced gastric compatibility and improved storage stability, addressing two significant limitations of conventional insulin therapy.

Table 7: Stability studies of formulation batches

Formulation	Initial pH	pH after 3 months
A1	3.29 ± 0.02	3.35 ± 0.01
A2	3.11 ± 0.01	3.36 ± 0.02
A3	3.31 ± 0.02	3.44 ± 0.01
B1	7.11 ± 0.02	7.12 ± 0.02
B2	6.78 ± 0.01	6.65 ± 0.01
B3	6.95 ± 0.02	6.25 ± 0.02

Conclusion and future research perspectives

The study successfully developed and evaluated insulin-loaded polymeric solid dispersions aimed at overcoming the challenges of oral insulin delivery, including enzymatic degradation and poor intestinal absorption. The use of amphiphilic and biocompatible polymers such as Soluplus[®], hydroxypropyl methylcellulose (HPMC), and ethyl cellulose resulted in solid

dispersion systems with high encapsulation efficiency and favourable micromeritic properties, supporting their suitability for oral administration. *In vivo* antidiabetic studies demonstrated that these formulations, particularly those containing Soluplus® and HPMC (B3), achieved significant and sustained reductions in blood glucose levels, highlighting their potential to provide effective glycaemic control comparable to or exceeding that of subcutaneous insulin. Additionally, the formulations maintained good physicochemical stability over three months and showed no evidence of hematological or severe biochemical toxicity, indicating an acceptable safety profile. These findings underscore the promise of solid dispersion technology as a viable strategy for oral insulin delivery, offering the potential to improve patient compliance, reduce injection-related complications, and enhance quality of life for individuals living with diabetes mellitus. Further research, including pharmacokinetic studies and clinical trials, is warranted to confirm these benefits and facilitate translation into clinical practice.

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Declaration of generative AI use

Authors declare that we did not use generative AI tools in the manuscript preparation process.

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