

Original Research Article

Development of Eudragit/gelatin-based microparticles for oral insulin delivery for diabetes treatment

Paul Akpa¹, Ben C Amadi², Calister E Ugwu², Josephat I Ogbonna^{2*}, Didacus N Nnamani³, Patience C Ugwuoke⁴, Chukwunweike I Okeke⁵, Mumuni A Momoh¹

¹Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, ²Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka, Enugu State, ³Department of Pharmaceutics and Pharmaceutical Technology, Dora Akunyili College of Pharmacy, Igbiniedion University Okada, Edo State, ⁴Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, ⁵Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nasarawa State, Nigeria

*For correspondence: **Email:** josephat.ogbonna@unn.edu.ng; **Tel:** +234-8063285077

Sent for review: 17 April 2025

Revised accepted: 22 July 2025

Abstract

Purpose: To prepare Eudragit® S100/gelatin-based insulin-loaded microparticles for improved oral delivery of insulin in diabetes management.

Methods: Eudragit® S100 and gelatin were employed in the formulation of insulin microparticles (EGel-MPs) for oral delivery at different ratios (0:1, 1:0, 1:1, 1:3, and 3:1 for Y1, Y2, Y3, Y4, and Y5, respectively) using double emulsion technique. A 1:1 ratio of Eudragit® S100 and gelatin microparticles not loaded with insulin (Y0) was used as a negative control, while aqueous insulin dispersion was used as a positive control. EGel-MPs were characterized for encapsulation efficacy (EE), scanning electron microscopy (SEM), and differential scanning calorimetry (DSC). In vitro drug release and in vivo antidiabetic activity in rats were also determined.

Results: Encapsulation efficacy ranged from 78.84 ± 0.24 to 89.43 ± 1.13 %, with Y4 as the highest, while SEM indicated spherical shapes and rough surfaces with pores. Particle sizes ranged from 6.22 ± 3.07 to 18.27 ± 0.02 μm . Batch Y3 depicted the broadest peak and lowest enthalpy, indicating an amorphous state and enhanced drug entrapment. In vitro insulin release exhibited a characteristic slow-release pattern, which may be attributed to mucoadhesiveness, leading to slow hydration, swelling, and gelling of particles within the buffer system. The in vivo antidiabetic activity was significantly better ($p < 0.05$) than that of both the negative and positive controls.

Conclusion: Bioavailability of orally delivered insulin has been improved using co-polymers of Eudragit® S100/gelatin microparticles (EGel-MPs). However, further studies are needed to quantify the bioavailability, rate of absorption and duration of glycaemic control.

Keywords: Eudragit® S100, Insulin, Gelatin, Antidiabetic activity, Microparticles

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Formulation scientists have been battling challenges in developing oral insulin delivery. Currently, parenteral insulin is the most common

drug product used to control Type I diabetes which has been taken as the most effective therapy [1]. This pharmaceutical parenteral dosage form has certain shortfalls such as erratic blood sugar levels associated with poor patient

compliance due to multiple dosing, inability to maintain a normal basal insulin rhythm, substantial tissue stress and aches, hypoglycemic episodes, and so on. Development of oral insulin would be the preferred option to prevent these challenges in order to improve insulin efficacy. To achieve effective oral insulin therapy, functional carriers must be designed to protect insulin from gastrointestinal enzymes and pH [2]. One of the strategies is cross-linking of different polymers with unique properties. The cross-linker should possess good muco-protective and pH-responsive properties [3]. Cross-linking insulin into a polymeric matrix has incomparable merits over conventional dosage forms. The polymeric matrix keeps the encapsulated medicine in a specific area in the body, allows for extended contact with tissue, and promotes pharmacological efficacy. This strategy is required because localizing the medicine at a particular location of absorption and channeling the drug across the intestinal epithelial barrier are the key issues associated with the low bioavailability that frequently plagues oral insulin delivery [4].

pH-sensitive medicines, engrafting polymers of methacrylic acid, sodium alginate, and polyethylene glycol, have been extensively studied for oral insulin delivery. Eudragit® has recently received a lot of attention for its potential to preserve peptide and protein medications for oral administration. Some advantages of Eudragit®, such as biocompatibility, mucoadhesive characteristics, and permeation-enhancing effects, have been reported [5]. Gelatin is another carrier polymer that is derived from the collagen of animals and contains peptides. It has muco-adhesive properties owing to its swellability and is used to control the release of active ingredients. Gelatin in combination with biopolymers, such as chitosan and polyvinyl alcohol, has been used as drug carrier [6].

Double microemulsion method of preparation of microparticles is a strategic technique that facilitates the formulation of oral insulin. This is a thermodynamically stable system that assists in the delivery of some sensitive moieties. The system prevents degradation of drug molecules, has ease of preparation, and high encapsulation efficiency in addition to other advantages [7]. This study aimed to take advantage of gelatin's bio-adhesive properties and Eudragit® S100's protective coating properties to boost the entrapment of insulin in the polymers' core, resulting in a more stable formulation with a greater protective role against acidic and enzymatic degradation in the GIT environment.

EXPERIMENTAL

Materials

Human recombinant insulin (Elly Pharm. Ltd., India), Eudragit®S100 (Evonik, Darmstadt, Germany), paraffin oil (Moko Pharm. Ltd, Nigeria), Gelatin (Yasin Gelatin Pvt. Ltd, India), Span®60 (BDH, Poole, England), Acetone (Merck, Germany), Glucometer (Accu-Check®, USA) and insulin (Eli Lilly, Nigeria), and other reagents were used without further treatment.

Preparation of microparticles

Double emulsion process was used to generate Eudragit® S100/gelatin microparticles (EGel-MPs) containing insulin, as shown in Table 1 [8]. Using 20 mL of distilled water, a 10 %w/v homogeneous mixture of 1 g Eudragit® S100 and 1 g of gelatin (1:1) was prepared. This was sonicated for 1 hour at 100 rotations per minute (rpm) with an Ultrasonic probe (Athena Technology, Virginia, USA), then sonicated for another 10 minutes after adding 10 mL of insulin. Each dispersion was poured into a 100 mL beaker containing 10 mL of liquid paraffin while being stirred with a magnetic stirrer set at 300 rpm. A 0.4 g of Span® 60 was dispersed in 1 mL of ethanol, mixed into the dispersion, being swirled at the same speed with a magnetic stirrer. The dispersion was gently poured into 200 mL of acetone kept at 2 °C and mixed at a slower speed for about 10 minutes before being placed in an ice jar to reduce the increase in temperature. The particles were recovered using filtration with filter paper and rinsed many times with acetone to eliminate any remaining liquid paraffin. The organic solvent was allowed to evaporate at room temperature, and the formulation was made up to 100 mL with distilled water. The microparticles were split into two parts: one was freeze-dried, while the second one was kept at 10 °C for further investigation. The method was repeated with various combinations of Eudragit® S100 and gelatin (0:1, 1:0, 1:1, 1:3, and 3:1). The microparticle compositions were kept in sealed plastic bottles, away from light, until they were used.

Percentage recovery (Y; %)

After lyophilization, the moisture-free dried Eudragit® S100/gelatin microparticles (EGel-MPs) from every batch were measured in order to determine the yield (Y) of the microparticles. Equation 1 was used to calculate the percentage yield.

Table 1: Composition of Eudragit® S 100-engrafted gelatin insulin microparticles

Batch	Gel (g)	E.S (g)	I (100 IU/mL)	Liq. P (mL)	Span®60 (g)	W (mL)
Y0	1	1	0	1	0.4	100
Y1	0	1	5	1	0.4	100
Y2	1	0	5	1	0.4	100
Y3	1	1	5	1	0.4	100
Y4	1	3	5	1	0.4	100
Y5	3	1	5	1	0.4	100

Y0 – Y5 (contain Eudragit®S100: gelatin ratio 1:1, 0:1, 1:0, 1:1, 1:3, and 3:1, respectively); Gel (gelatin); E.S (Eudragit® S100); I (insulin); Liq. P (liquid paraffin); W (water)

$$Y (\%) = (W1/W2+W3)100 \dots\dots\dots (1)$$

Where W1 is the weight of recovered microparticles, W2 is drug weight, and W3 is weight of polymer plus other excipients.

Determination of entrapment efficiency

The entrapment efficiency (EE) and loading capacity (LC) of EGel-MP preparations were calculated by centrifuging the microparticles at roughly 5000 rpm for half an hour, and the supernatant was analyzed for non-encapsulated insulin content using a spectrophotometer [9]. Equations 2 and 3 were used to compute EE and LC, respectively.

$$EE (\%) = (Ti-Fi/Ti) 100 \dots\dots\dots (2)$$

$$LC (\%) = (Ti-Fi/Mn)100 \dots\dots\dots (3)$$

Where Ti is total amount of insulin, Fi is free insulin in supernatant Mn is mass of microparticles.

Characterization of microparticles

Scanning electron microscopy

Scanning electron microscopy (Hitachi Japan, Model 3400N) was used to assess the size and surface morphology of Eudragit® S100/gelatin-engrafted insulin-loaded microparticles. The microparticle suspension (10 µL) was put on a glass slide and dried at 25 ± 0.5 °C.

These specimens were mounted to the stub and gold-sputtered to reduce the charging effects before scanning with a 20 kV acceleration voltage. These specimens were mounted to the stub and gold-sputtered to reduce the charging effects before scanning with a 20 kV acceleration voltage.

Particle size and morphology analysis

The shape and size of the microparticles were measured utilizing computerized image

processing using a polarized light microscope. In brief, one drop of insulin-loaded MPs from each batch was placed on a slide (Mansfield, Germany).

The specimen was then covered with a cover slip and examined using a polarized light microscope. Particle morphologies were studied using the microscope's software, and photomicrographs were produced at x1000 magnification. The particle sizes were measured, and an average time was recorded.

Polydispersity Index

The mean diameter and polydispersity index of drug-loaded MPs were determined using a Zetasizer (Nano-ZS, Hitachi, Japan). Before photon correlation spectroscopic (PCS) analysis, all samples were diluted with a constant amount of double-distilled water to achieve the desired intensity of scattering.

Differential Scanning Calorimetry

A calorimeter (Netzsch DSC 204 F1, Germany) was used to carry out thermal analysis and measure changes in heat capacity of pure drug, microparticles, and excipients. To evaluate thermal behavior, 5 mg of each material was placed into an aluminum pan, sealed hermetically, and heated at 5 °C/min from 0 and 300 °C. An empty pan was used to establish the baselines and used as a reference to correct all of the thermograms.

Stability study

pH stability

The pH and organoleptic evaluations of microparticle suspension were used to determine changes in formulation quality. The pH of the MPs was measured on the first, seventh, and twenty-eighth days after formulation to assess potential pH variations caused by API or excipient degradation. The pH of every batch was measured potentiometrically using a

pH meter, and the mean pH was determined in triplicate after calibration with a pH 7.0 standard buffer.

Re-dispersibility and Sedimentation rate

The formulations were rotated vertically and the amount of oscillation required to re-disperse the formulation was measured quantitatively. This was estimated at 24, 168, and 672 hours. The formulations were left standing and undisturbed, and sedimentation rates (Sr) were calculated using Eq 4.

$$Sr = (TL - SL / TL) 100 \dots\dots\dots (4)$$

Where TL is total formulation length, SL is supernatant length (uppermost clear zone length).

In vitro release studies of Eudragit® S100/gelatin engrafted insulin microparticles

The drug release patterns of insulin-loaded microparticles *in vitro* were studied using a membrane dialysis method. A 1.5 mL formulation was applied to a dialysis membrane (MWCO 8000 - 10000, Spectrum Labs, Netherlands) that had been prepared with phosphate buffer overnight, along with 1 mL of buffer dilution (pH 7.2). The membrane was immersed in 500 mL of pH 7.2 phosphate buffer and agitated with a drug dissolving equipment set to 100 rpm and 37 ± 1 °C (Glass Agencies, India). A 5 mL sample was extracted at 30 minutes, 1, 2, 4, and 8 hours. The aliquots were withdrawn and replaced with 5 mL of the fresh buffer solution. The withdrawn samples were then filtered using a 0.22 µm filter (Millipore, USA). The amount of insulin in each sample in the aliquot was measured using Lowry's test at 271 nm with a UV Spectrophotometer (Spectrum Lab, the Netherlands). All dissolution experiments were done three times. The total quantity of insulin released at different times was calculated using the method described above and plotted against time to obtain the release profile. The above method was repeated with the remaining batches.

Determination of the mechanism and drug release kinetics

To evaluate the insulin release kinetics and mechanisms of microparticles, *in vitro* insulin release values were fitted into 4 different release models: zero order, first order, Higuchi and Korsmeyer-Peppas models as illustrated in Equations 5 – 8 [9]. The R² values were calculated for the following plots: zero-order

(cumulative drug release versus time), first-order (log cumulative of % drug remaining vs. time), Higuchi (cumulative % drug release vs. square root of time), and the integral form of Higuchi, log. Cumulative % drug release vs. log time and Korsmeyer-Peppas (log fraction of drug release versus log time).

$$Q_t = K_0 t \dots\dots\dots (5)$$

$$\text{Log } Q_0 = K_1 t^{2.303} \dots\dots\dots (6)$$

$$Q = K_2 t^{1/2} \dots\dots\dots (7)$$

$$\frac{M_t}{M_\infty} = K_3 t^n \dots\dots\dots (8)$$

Note: Q is the quantity of drug released at time t, Q₀ is the initial quantity of the drug in the matrix, K₀, K₂, and K₃ are zero-order, first-order, Higuchi and Korsmeyer -Peppas kinetic constants. M_t/M_∞ is the fraction of drug released at time t; n is the diffusion exponent indicative of the mechanism of drug release from the matrix.

In vivo study in diabetic rats

Induction of diabetes

Prior to the start of the experiment, the Animal Committee on Ethics at the University of Nigeria, Nsukka, approved the procedure for the use of animals (approval no. FVM-UNN-IACUC-2018-058). All experiments conformed to the International Ethical Standards for Use of Animals.

Wistar rats (males) weighing 98 - 110 g were confined for one week to adapt to the ambient temperature and relative humidity (22 ± 2 °C and 45 - 65 %) of the laboratory. Conventional meal (Feeds BC, Nsukka, Nigeria) was used to feed the rats, but with unlimited access to potable water. The lighting was set to a conventional 12-hour on, 12-hour off schedule. Diabetes was created in the rats with a single intraperitoneal administration of newly prepared alloxan solution (in citrate buffer) at the dose of 150 mg/kg body weight [10]. After 14 days, rats with fasting blood sugar levels of more than 250 mg/dL were chosen for the studies.

Antidiabetic test

The forty-two rats with diabetes were assigned randomly to six distinct groups, with each group housed in its own cage. The animals went without food for 12 hours prior to and during the testing period, though they were permitted to

drink freely. Rats were given different ratios of Eudragit® S100 - engrafted gelatin insulin microparticles intragastrically via gavage needle, with a dose of insulin of 100 IU/kg on the basis of the microparticles' total insulin content. The rats were divided into six groups in the following manner: Group 1 received formulation Y3 (Gelatin: Eudragit® S100; 1:1); Group 2 received formulation Y4 (Gelatin: Eudragit® S100; 1:3); Group 3 received formulation Y2 (Gelatin: Eudragit® S100; 1:0); Group 4 (Control) received formulation Y0 (placebo); Group 5 received positive control (aqueous insulin dispersion per oral) and Group 6 (Control 3) received subcutaneous insulin. The animals fasted for 12 hours before the beginning of the trial. The rats were subsequently treated according to the procedure, and blood glucose concentrations were measured at specified periods, 0.5, 1, 3, 6, 9, 12, and 24 hours.

Statistical analysis

The Instant 2.00 Macintosh application (California) was used to perform one-way analysis of variance, Dunnett multi-comparison test and Duncan post hoc test. A p-value < 0.05 indicated a significant difference between means.

RESULTS

Encapsulation efficiency, drug loading capacity, and percentage yield

The MPs showed EE (%) range from 78.84 ± 0.24 - 89.43 ± 1.13 %. Y4 (containing Gelatin: Eudragit® S100 ratio 1:3) exhibited the highest EE % (89 %). Increasing carrier concentration considerably ($p < 0.05$) increases EE (%).

Percentage recovery

The method directly affects the formulation's percentage yield. Table 2 shows that the loaded formulation had the highest yield. A high percentage of MPs recovered from formulations

(≥ 80 %) indicates a reliable formulation procedure.

DSC findings

The DSC results showed insulin, Eudragit® S-100, and gelatin had points at 138.47, 65.03, and 107.41 °C, respectively, with corresponding peaks at -97.71, -1.16, and -2.10. Thermal peaks for the formulation (Figure 1) containing gelatin and Eudragit® S100 coded as: Y0, Y1, Y2, Y3, Y4, and Y5 showed thermal endothermic peaks 147.71, 147.48, 145.44, 143.68, 148.26, and 143.94 °C with corresponding enthalpies of -77.55, -0.48, -49.01, -114.50, -41.17, and -49.32, respectively. However, formulation Y3 showed the broadest peak with the lowest enthalpy, unlike others that exhibited sharper peaks. The sharpness and symmetry of the endothermic peaks indicate high-purity matrices, as shown in formulations Y0, Y1, Y2, Y4, and Y5. The broad peaks, as in formulation Y3, indicate an amorphous state.

Scanning electron microscopy, particle size and polydispersity index

Scanning electron microscopy (SEM) study of Eudragit®S100/gelatin engrafted microparticles (EGel-MPs) prepared with Eudragit® S100: gelatin ratios 1:1 (Y3) and 1:3 (Y4) is presented in Figure 2. The surface morphology of the unloaded formulation Y0 (Figure 2 i) was observed to be uniformly dispersed with pores. The surface morphology of the loaded formulation Y4 (Figure 2 ii) was a mixture of spherical shapes and rough surfaces with pores. Particle size growth was also observed in Y4. These structural deformations are a result of drug diffusion and the lyophilization processes. The particle sizes of EGel-MPs as presented in Table 1 ranged from 6.22 ± 3.07 to 18.27 ± 0.02 µm. It was discovered that particle sizes increased with increase in the concentration of the polymer, such that particle sizes of Y1 (containing Gelatin: Eudragit® S 100) 0:1 were 7.63 ± 3.08 µm, while in a higher concentration of Y4, it was doubled (14.45 ± 1.12 µm).

Table 2: Encapsulation efficiency, drug loading, % yield, and particle sizes of EGel MPs

Batch	EE (%)	LC (%)	Yield (%)	Particle size (µm)
Y0	-	-	88.35 ± 1.12	6.22 ± 3.07
Y1	85.49 ± 0.49	7.45 ± 0.24	86.34 ± 1.83	7.63 ± 3.08
Y2	78.84 ± 0.24	2.23 ± 0.96	76.43 ± 2.93	13.47 ± 2.04
Y3	89.24 ± 0.64	6.43 ± 0.37	89.47 ± 1.34	7.04 ± 2.03
Y4	89.43 ± 1.13	6.45 ± 1.63	83.36 ± 1.47	14.45 ± 1.12
Y5	78.93 ± 1.93	7.1 ± 0.74	87.37 ± 1.93	18.27 ± 0.02

Key: Y0, Y1, Y2, Y3, Y4, and Y5 are gelatin-Eudragit® S100 polymer microparticles in the ratio of 1:1 (unloaded), 0:1 (loaded), 1:0 (loaded), 1: 1 (loaded), 1:3 (loaded) and 3: 1 (loaded), respectively

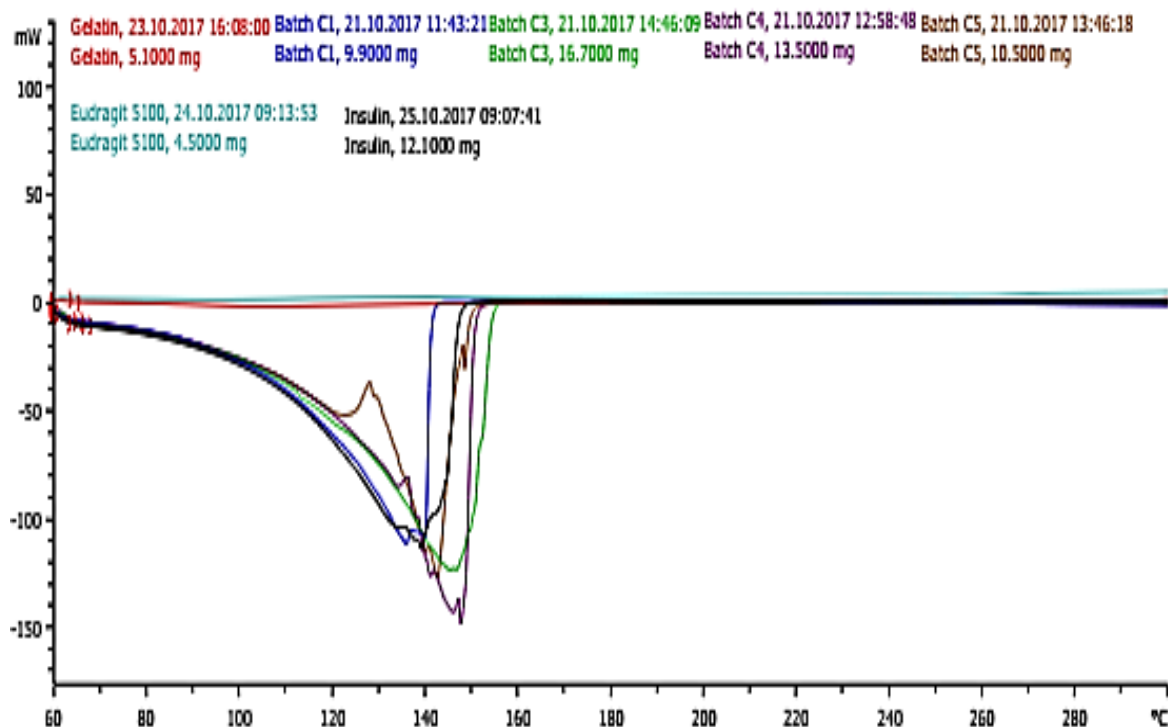


Figure 1: DSC thermograms of Eudragit® S 100, insulin, gelatin, and microparticles. Batches C1, C3, C4, C5 are Gelatin-Eudragit® S100 polymer microparticles in ratios of 0:1, 1:1, 1:3 and 3:1, respectively

Moreover, the type of polymer employed is a factor affecting particle sizes. Eudragit® S100 MPs exhibited lower particle sizes, unlike gelatin MPs. Loaded formulation Y3 ($7.04 \pm 2.03 \mu\text{m}$) showed no significant change in particle sizes from unloaded formulation Y0 ($6.22 \pm 3.07 \mu\text{m}$). The PDI of the microparticles are 0.884 ± 0.01 , 0.301 ± 0.15 , and 0.109 ± 0.27 for formulations Y1, Y3, and Y4, respectively. PDI decreased as the concentration of Eudragit®S100 increased in

the formulation. Therefore, the PDI was poor, with the highest value for Y1 (Gelatin: Eudragit® S100 ratio 0:1) being 0.884. The implication of this may be an increase in the residence time in the stomach, which might lead to the degradation of the polymeric microparticles and subsequently, the insulin drug. Thus, MPs-Y3 and Y4 might show better biodistribution and subsequently bioavailability than Y1.

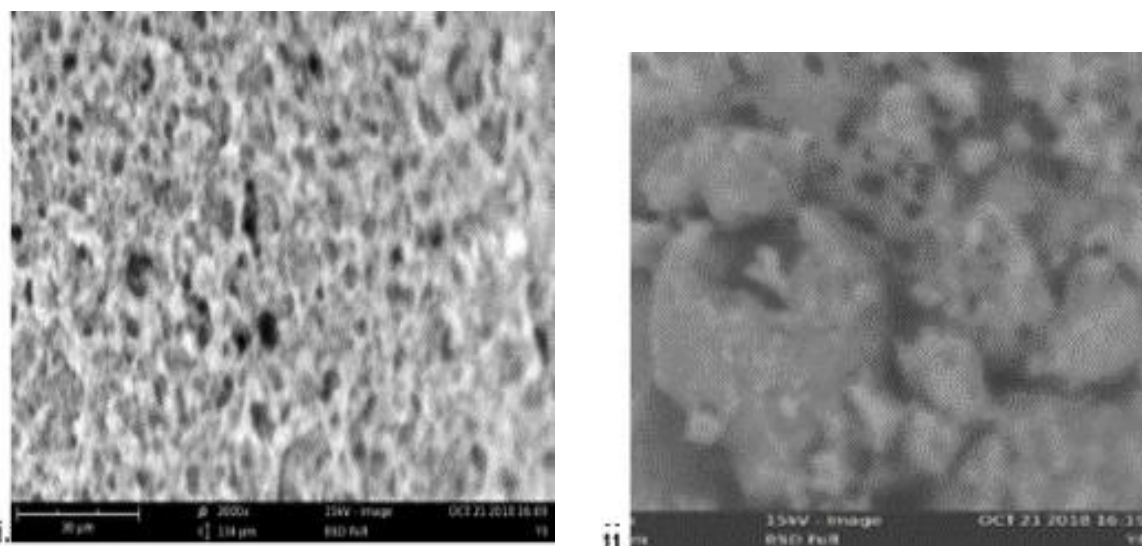


Figure 2: SEM of unloaded (Y0) and loaded (Y4) microparticles. **Key:** i and ii are Gelatin-Eudragit® S100 microparticles formulated with the ratio of 1: 1 (Y0) and 1:3 (Y4), respectively

Stability

The pH of EGel-MPs ranged from 7.3 - 9.5 (Figure 3). In these formulations, there was a gradual increase in pH from 1st day to the end of the 28th day. The presence of moisture may induce microbial activity, causing a change in pH. This change in the pH may affect the insulin stability because it is higher than the established stable pH of insulin, which is 7.3. The active ingredient's denaturation results in a loss of potency. The incorporation of preservatives in these MPs during formulation is recommended, or the product should be freeze-dried.

Egel-MPs' *in vitro* drug release and kinetics

Insulin release *in vitro* from the Gelatin-Eudragit® microparticles (EGel-MPs), as presented in Figure 5, ranged from 19 – 98 %. There was gradual drug release in all formulations except formulation Y3, which showed a burst effect before gradual drug release. Other formulations have typical sustained release patterns such that most of the formulations have not released up to 40 % within the experimental time of 8 h, even though all showed EE $\geq$ 78 %. The kinetics of drug release from the formulations was investigated using important release models such as first order, Higuchi's, zero order, and Korsmeyer-Peppas.

Table 2 shows the computed R^2 coefficients of

the trendlines for several release models, revealing a very strong fit with the data from experiments and the Korsmeyer-Peppas concept.

The Korsmeyer-Peppas model for EGel-MPs yielded n values that were below 0.45, implying that the microparticles released the entrapped drug via Fickian diffusion by erosion of the surface.

In vivo drug activity

Figure 6 shows the effectiveness of two formulations of insulin-loaded microparticles prepared with Gelatin: Eudragit® S100 1:1 (Y3) and 1:3 (Y4) on the alloxan-induced diabetic rats. The orally and subcutaneously (SC) administered commercial insulins acted as the positive controls, and the unloaded formulation Y0 was employed as a negative control. All the groups received an equivalent of 100 IU/kg of insulin in order to obtain a direct comparison of the dose-response in the experiment. In both positive controls, SC insulin decreased blood sugar levels (BSL) significantly ($p < 0.05$) within 1 hour and reached 45 mg/dL at 2 hours post-injection. However, the BSL then increased to 80 mg/dL after 12 hours of injection. The formulation Y0 (placebo) treated diabetic rats showed no sign of a drop in their BSL, ruling out the possibility of the polymers having hypoglycemic effect.

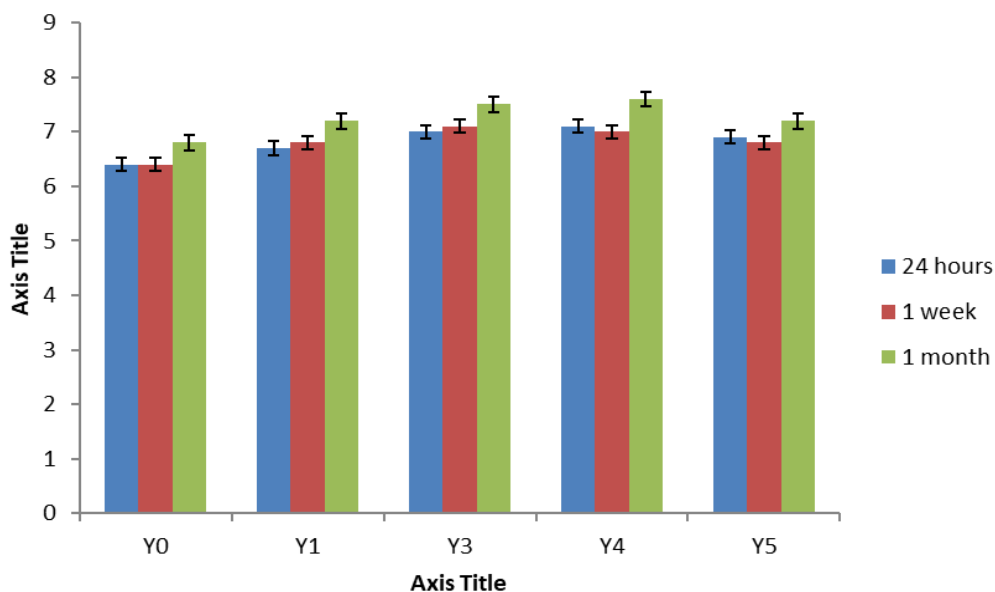


Figure 3: pH of Eudragit® S100/gelatin engrafted insulin microparticles. **Key:** Y0, Y1, Y2, Y3, Y4, and Y5 are gelatin-Eudragit® S100 polymer microparticles in the ratio of 1: 1 (unloaded), 0:1 (loaded), 1:0 (loaded), 1:1 (loaded), 1:3 (loaded), and 3:1 (loaded), respectively

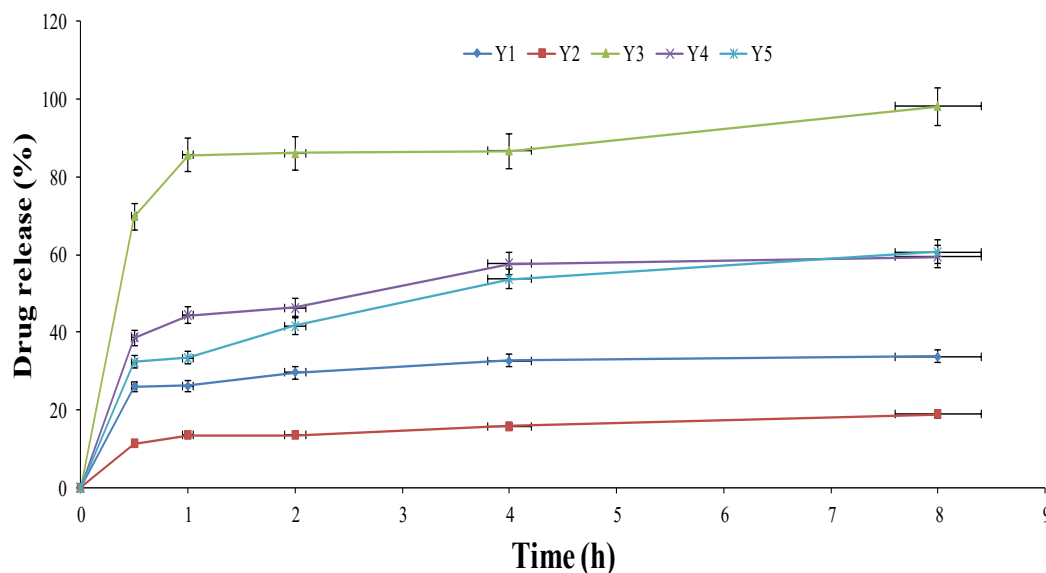


Figure 4: *In vitro* release of Eudragit®-gelatin engrafted insulin microparticles. **Key:** Y1 – Y5 (microparticles containing Gelatin: Eudragit® S100 ratios (0: 1, 1:0, 1:1, 1:3, and 3:1, respectively), (n = 3)

Table 3: Mathematical models for drug release mechanisms of EGel-MPs

Batch	Zero-order R ²	First order R ²	Higuchi's R ²	Korsmeyer's Peppers R ²	n
Y1	0.8273	0.8273	0.9191	0.9421	0.108
Y2	0.9527	0.9527	0.967	0.9409	0.174
Y3	0.4168	0.4168	0.3856	-	-
Y4	0.8187	0.8187	0.9111	0.9505	0.163
Y5	0.9096	0.9096	0.9662	0.9575	0.251

Y1 – Y5 (microparticles containing Gelatin: Eudragit® S100 ratios (0:1, 1:0, 1:1, 1:3, and 3:1), respectively), (n = 3)

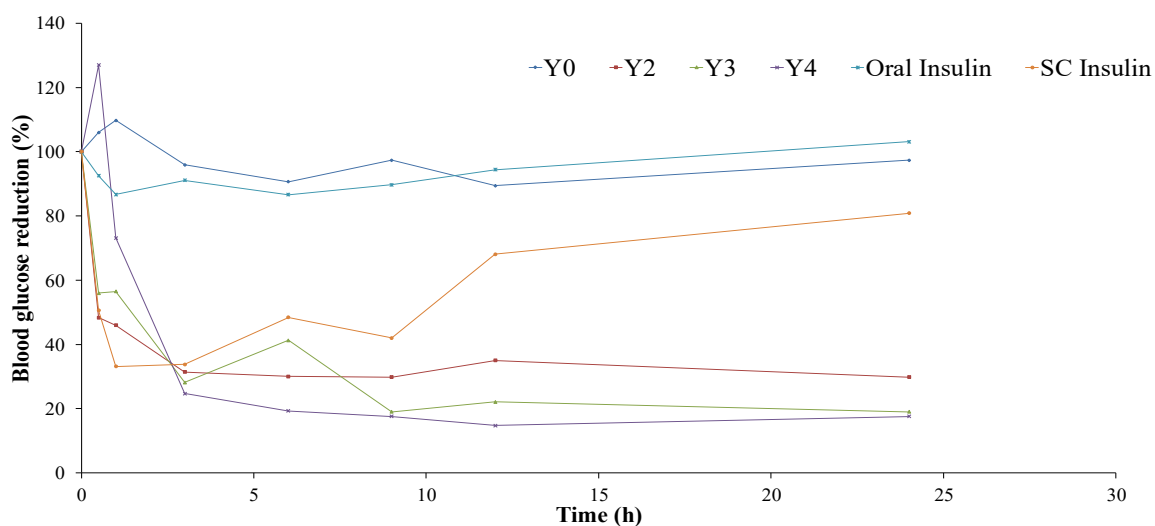


Figure 5: Percentage decrease of the blood glucose level in Wistar male rats treated with insulin-loaded microparticles. **Key:** Y2 – Y4 (microparticles containing insulin-loaded Gelatin: Eudragit® S100 ratios 1:0, 1:1, and 1:3, respectively), Y0 (microparticles containing unloaded Gelatin: Eudragit® S100 ratios 1:1; n = 7)

Formulations F3 (containing Gelatin: Eudragit® 1:1) and F4 (containing Gelatin: Eudragit® 1:3) exhibited significant ($p < 0.05$) % reduction in BSL to 25 mg% for over 24 h. In the first half

hour post-administration of formulation Y4, the BSL increased continuously before reducing in the second half of the hour. Since Eudragit® S100 is pH sensitive, this could be due to

resistance in drug release at lower pH and partly due to the swelling, gelling, and ionization of the polymer at an appropriate pH medium. This phenomenon was not observed in formulation Y3 as it had a lower concentration of Eudragit® S100. Also, the opposing charge created by Eudragit®/gelatin and the mucus membrane of the GIT facilitated insulin absorption through Peyer's spots.

DISCUSSION

The goal of any pharmaceutical delivery device is to transport the entrapped drug to the tissues that are targeted with little or no negative impacts on tissues or body functions [11]. High yields ($\geq 80\%$) of MPs indicate a reliable formulation procedure. The percent yield of MPs is directly proportional to the procedures used [12]. Table 1 shows that the MPs produced good yields. Formulation Y3 produced the highest percentage yield. In storing MPs under refrigerated conditions ($4\text{ }^{\circ}\text{C}$), the activities of micro-organisms were low but were not eliminated. In the MPs of Eudragit® S100, there was a reduced activity of micro-organisms since the pH could not create a favourable condition for microbial growth. Moreover, there were no significant changes in the appearance and odour of the formulations, especially in Y4 (containing gelatin: Eudragit® 1:3). This shows that an increase in the concentration of Eudragit® S100 protected the formulation from micro-organisms and acted as a preservative, inhibiting microbial growth. However, further study may be conducted over a long period to determine the stability. It has been reported that some microemulsions containing peptides and proteins formulated using the double emulsion technique exhibited a substantial tendency for the drugs to move to the aqueous medium during the release study, thus concentrating at the surface, which results in a burst release effect. This is consistent with a previous report [13]. This burst release effect is of clinical importance as a loading dose if it is needed in the management of some ailments, such as diabetes.

Insulin release from microparticles was pH-driven and took into account the rate of polymer degradation. As a result, the erosion of the polymer caused the release of an encapsulated protein, which then diffused through the channels created by this process [14]. Erosion has been shown to generate molecules that are able to interact with the entrapped protein, resulting in denaturation [15]. Slow release from formulations may also be attributed to slow hydration rate, swelling, and gelation of the formulation in phosphate buffer. These formulations will be

patient-friendly since the administration will be once in 24 h. Particle size is another factor that may affect drug release. Formulation Y3 depicted the highest insulin release (98 %) after 8 h of the experiment and exhibited the smallest particle sizes ($7.04 \pm 2.03\text{ }\mu\text{m}$). It has been reported that the lower the particle sizes, the higher the drug release [16].

Previous reports have shown that the synergistic mucoadhesive properties of hybridized polymers (Eudragit® and gelatin) and their cationic natures allow insulin to be absorbed via lymphatic uptake through the M cells of the Peyer's patches in the gastrointestinal tract, leading to an increase in the glycemic effects of insulin-loaded nanoparticles [17]. Finally, the observed burst insulin release effect in F3 supplied the immediate loading dose to suppress the BSL. The % blood glucose level reduction observed with orally administered EGel-MPs in diabetic rats was achieved through the prominent mucoadhesive properties of the hybridized polymers, which are cationic and provide a sheath over insulin in GIT harsh environment of the GIT absorptive site. Thus, the oral administration of insulin is a possibility through the right choice of polymers and formulation technology.

CONCLUSION

Eudragit® S100/gelatin engrafted insulin microparticles (EGel-MPs) have been successfully prepared for oral insulin delivery in order to enhance the insulin payload in the inner core of co-polymers and achieve a more stable formulation with a greater protective role against acidic and enzymatic degradation in the GIT. The extended insulin release maintains plasma concentration over a significant period.

DECLARATIONS

Acknowledgement/Funding

The work was supported materially by Prof. Mumuni Momoh.

Ethical approval

The University of Nigeria, Nsukka's Animal Ethics Committee gave its approval for the use of these animals in these studies (approval no. FVM-UNN-IACUC-2018-058).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Momoh A. Mumuni, Paul Akpa, and Ben Amadi conceptualized the research, designed the experiments. Paul Akpa, Ben Amadi, and Momoh A. Mumuni performed the experiments and collected the data. Calister E. Ugwu, Josephat I. Ogbonna, and Chukwunweike Ikechukwu Okeke analyzed and interpreted the data. Josephat I. Ogbonna, Mumuni A. Momoh, Calister E. Ugwu, Ugwuoke Chinenye Patience, Ben Amadi, and Didacus Nnabuike Nnamani wrote both the original draft and the final copy of the paper.

REFERENCES

1. Syed FZ. Type 1 diabetes mellitus. *Ann Intern Med* 2022; 175(3): ITC33 - 48. <https://doi.org/10.7326/AITC202203150>
2. Alfa J, Ben A, Buxaderas E, Akpa P, Hanifah A, Oseni OM, Kenechukwu FC, Mumuni MA, Diaz DD. Development and evaluation of PEG-gelatin-based microparticles to enhance the oral delivery of insulin. *Curr Pharma Des* 2024; 30(24): 1939 - 1948. <http://dx.doi.org/10.2174/0113816128309449240527053640>
3. Shetty SS, Halagali P, Johnson AP, Spandana KA, Gangadharappa HV. Oral insulin delivery: Barriers, strategies, and formulation approaches: A comprehensive review. *Int J Biol Macromol* 2023; 242: 125114. <https://doi.org/10.1016/j.ijbiomac.2023.125114>
4. Jain SK, Jain AK, Rajpoot K. Expedition of Eudragit® polymers in the development of novel drug delivery systems. *Curr Drug Deliv* 2020; 17(6): 448–469. <https://doi.org/10.2174/1567201817666200512093639>
5. Momoh MA, Jackson TC, Musiliu AO, Chimaobi KF, Darlington YC, Ofokansi KC. Microspheres of insulin-Eudragit complex: formulation, characterization and in vivo studies. *Afr J Pharm Pharmacol* 2017; 11(29): 327–341. <https://doi.org/10.5897/AJPP2017.4796>
6. Mathew SA, Arumainathan S. Crosslinked chitosan-gelatin biocompatible nanocomposite as a neurodrug carrier. *ACS Omega* 2022; 7(22): 18732 - 18744. <https://doi.org/10.1021/acsomega.2c01443>
7. Ogbonna JI, Momoh MA, Agbo CP, Abdulmumin H, Chukwu CC, Alfa J, Aminu N, Oyeniyi J, Okino US, Kenechukwu FC, Alfred-Ugbenbo D. Development of double-coated microparticles for improved oral insulin delivery in diabetes management. *Trop J Pharm Res* 2025; 24(2): 141 - 151. <https://doi.org/10.4314/tjpr.v24i2.2>
8. Builders PF, Kunle OO, Okpaku LC, Builders MI, Attama AA, Adikwu MU. Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. *Eur J Pharm Biopharm* 2008; 70(3): 777–783. doi: 10.1016/j.ejpb.2008.06.021
9. Paarakh MP, Jose PA, Setty CM, Peter Christopher GV. Release kinetics—concepts and applications. *Int J Pharm Res Technol (IJPRT)* 2018; 8(1): 12–20.
10. Etuk EU. Animal models for studying diabetes mellitus. *Afr J Biomed Res* 2010; 13(3): 161–167.
11. Jain KK, Chibuzor Amadi B, Akpa P, Buxaderas E, Moglie YF, Alfa J, Romanus O, Nnabuike Didacus N, Chimaobi Kenechukwu F, Audu Mumuni M, et al. Development of gelatin-sodium alginate microparticles for oral insulin delivery. *Afr J Pharm Res Dev* 2024; 16(3): 105–116.
12. Agrawal GR, Wakte P, Shelke S. Formulation, physicochemical characterization and in vitro evaluation of human insulin-loaded microspheres as potential oral carrier. *Prog Biomater* 2017; 6: 125–136. <https://link.springer.com/article/10.1007/s40204-017-0072-z>
13. Mumuni MA, Kenechukwu FC, Ernest OC, Oluseun AM, Abdulmumin B, Youngson DC, Kenneth OC, Anthony AA. Surface-modified mucoadhesive microparticles as a controlled release system for oral delivery of insulin. *Heliyon* 2019; 5(9): e02366. doi: 10.1016/j.heliyon.2019.e02366.
14. Shi M, McHugh KJ. Strategies for overcoming protein and peptide instability in biodegradable drug delivery systems. *Adv Drug Deliv Rev* 2023; 199: 114904. <https://doi.org/10.1016/j.addr.2023.114904>
15. Lin X, Yang H, Su L, Yang Z, Tang X. Effect of size on the in vitro/in vivo drug release and degradation of exenatide-loaded PLGA microspheres. *J Drug Deliv Sci Technol* 2018; 45: 346 - 356. <https://doi.org/10.1016/j.jddst.2018.03.024>
16. Momoh AM, Ugwu EC, Nafiu A, Kenechukwu CF, Adedokun OM, Usman M, Barikisu A, Oyeniyi YJ, Ofokansi KC, Attama AA, et al. Mucin-grafted polyethylene glycol microparticles enable oral insulin delivery for improving diabetic treatment. *Appl Sci* 2020; 10: 2649. <https://doi.org/10.3390/app10082649>
17. Souto EB, Souto SB, Campos JR, Severino P, Pashirova TN, Zakhharova LY, Silva AM, Durazzo A, Lucarini M, Izzo AA, et al. Nanoparticle delivery systems in the treatment of diabetes complications. *Molecules* 2019; 24(23): 4209. doi: 10.3390/molecules24234209.