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Original Research Article

Development of double-coated microparticles for improved oral insulin delivery in diabetes management

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Abstract

Purpose: *To formulate double-coated insulin-loaded polymer-based microparticles (MPs) for oral delivery of insulin.*

Methods: *Different formulations of insulin-loaded MPs were prepared using polyethylene glycol 4000 (PEG 4000) and chitosan as primary coat and Eudragit® RL 100 as secondary coating agents. Physicochemical characterization, in vitro drug release, toxicological, and in vivo studies in diabetic rats were performed, and the results of the orally administered MPs were compared with those of subcutaneously administered Humulin®.*

Results: *The developed MPs showed good physicochemical characteristics. In vitro release studies showed that all batches of MPs exhibited sustained insulin release in 12 h with the highest insulin release achieved by MPs formulated using PEG 4000 as primary coating. In vivo, the orally administrated MPs containing 2 % chitosan achieved a reduction in blood glucose level from 100 mg/dL to 15.8 mg/dL after 10 h, compared to subcutaneously administered Humulin® which was 100 mg/dL to 20.60 mg/dL after 24 h. The MPs reduced blood urea (76.25 - 43.21 mg/dL) better than Humulin® (76.25 - 73.11 mg/dL), hence, may prevent development of insulin resistance and/or defective insulin release.*

Conclusion: *The effects of these formulations on blood glucose were comparable to subcutaneously administered Humulin® in diabetic rats. However, there is need to optimize these polymer blends for improved effectiveness, as well as study the long-term stability of these formulations.*

Keywords: *Diabetes, PEG 4000, Chitosan, Double coating, Microparticles, Insulin*

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INTRODUCTION

One of the modern era's therapeutic miracles is the discovery of insulin. This discovery is helping to save millions of lives and maintaining the health of people living with diabetes mellitus worldwide [1]. But its applicability has been hampered significantly over the years by its mode of administration, amidst other challenges. Therapeutic insulin is typically administered via subcutaneous injection [2]. Several non-invasive routes for insulin delivery that have been investigated include intrapulmonary, oral, ocular, nasal, buccal and transdermal systems [3]. Oral administration of drugs is generally preferred especially in chronic diseases such as diabetes, which requires life-long therapy due to the advantages it confers such as being painless and convenient in administration and requiring no skill. Regrettably, problems related to poor absorption, high proteolytic degradation and low bioavailability have been associated with oral insulin administration [2,3].

Microencapsulation (i.e. a polymer membrane or lipid encapsulating a drug), is trending in research as it protects some sensitive molecules from the harsh environment of gastrointestinal tract (GIT) after oral administration. More importantly, it allows manipulation of the release of drug, bypasses the activities of intestinal enzymes and the drug is targeted to a specific site. Micro/nano encapsulation of insulin using pH-sensitive polymers could prove beneficial in the search for a suitable oral delivery system for insulin. Furthermore, the micro or nano-size range of such formulations may enhance the transport of insulin across the GIT epithelium. These two effects are likely to improve the oral bioavailability of insulin. Previous works have succeeded in encapsulating insulin within mucin-grafted polyethylene glycol-based microparticles which were able to reduce blood glucose levels significantly in diabetic mice [4,5].
Also, some works have established that insulin microparticles could be formed either by precipitation in the presence of non-ionic polymers such as PEG 4000 or via complexation of insulin with chitosan. It has also been demonstrated that combining PEG 4000 and chitosan (CTS) brings about a synergistic effect and thus an improvement of the encapsulation efficiency (EE) of insulin in such MPs [6]. However, insulin-encapsulated CTS particles are usually pH-responsive, and readily protonate in acidic region of the GIT and prematurely release insulin in an unfavourable region thereby degrading the insulin before its absorption [7].

In order to prevent this, insulin MPs formulated in this study were enclosed within Eudragit® RL 100 polymer since this polymer is not soluble in acidic pH but at pH 7.4 and provides a more controlled release of the drug [4]. The controlled and sustained release of insulin from these formulations is hoped to prevent dose dumping and hypoglycaemia when used clinically, as opposed to only the hydrophilic polymers used alone [6].

In this study, insulin-loaded microparticles were formulated using PEG 4000 and/or CTS as primary coating, and Eudragit® RL 100 as secondary coating by employing double emulsion solvent evaporation technique which may provide better protection than conventional emulsions [8].

EXPERIMENTAL

Materials

Humulin® 70/30 (Ely Lilly Pharmaceuticals Ltd), polyethylene glycol (PEG) 4000, chitosan (100, 000 KDa, with 85 – 95 % deacetylation; Wako Chemical Ltd, Japan), polyvinyl alcohol (PVA; Sigma-Aldrich, England), Phospholipon® 90G (P90G; Lipoid GmbH, Germany), Eudragit® RL 100, Poloxamer® 407 (P407; BDH, England), dichloromethane (DCM; Guangdong Sci-Tech Co. Ltd, China), alloxan monohydrate (Sigma Chemical Company, USA), Accu-Check® glucose meter (Roche Diabetes Care, Inc., USA). Other reagents utilized were of analytical grade and were used as procured without additional treatment.

Preparation of polymer-lipid mixture

The polymer-lipid binary mixture of 9:1 ratio of Eudragit®RL-100 and Phospholipon® 90G (P90G) was prepared by dissolving it in dichloromethane based on preliminary studies. In brief, 9.0g of Eudragit® RL 100 and 1.0 g of P90G were transferred into 200-mLbeaker containing dichloromethane and the mixture was stirred thoroughly until the polymer-lipid dissolved completely.

Preparation of microparticles loaded with insulin

Polymer-based microparticles loaded with insulin were developed using a double emulsion method. Humulin® vial was shaken well and 1 mL of the suspension was added to 10 mL of oil phase consisting of mixture of Eudragit® RL 100 and P90G in a ratio of 9:1. The first phase of the water-in-oil (w/o) emulsion was formed after homogenization of the above mixture was carried

out with Ultra-Turrax (T18 basic, IKA Germany) homogenizer at 4000 rotations per minute (rpm) for five rows of 10 s. Ten milliliters of 5, 10 or 20 % solution of PEG 4000 was placed in a clean 100 mL beaker and 5 mL of 2 % PVA was added to the beaker and stirred for 5 min at 100 rpm using a magnetic stirrer (BioCote® Stuart Hotplate stirrer). A 5 mL of 2 % Poloxamer® 407 (P407) was incorporated into the mixture and stirred for further 5 min at the same speed. The w/o emulsion obtained initially was then added in drops to a second mixture to form a double emulsion of water-in-oil-in-water (w/o/w). The preparation was left open to allow evaporation of the organic phase. The same procedure was followed for the batches formulated with 10 mL of 2, 4 or 6 % chitosan solution in 0.1 N acetic acid instead of PEG-4000, while for batches R1, R2 and R3, 10 mL of a 5, 10 and 20 % PEG 4000 solution and 4 mL of 2 % chitosan solution were both used to produce the w/o/w emulsion (Table 1).

Determination of particle size and morphology

The mean diameters and morphology of insulin-loaded MPs were analyzed using Moticam 4.0 digital camera (Moticam, China) attached to an optical digital light microscope (DLM; Leica Diestar, Germany). Images of the microparticles were captured on a slide at × 400 magnification and particle sizes were measured. All measurements were performed in triplicates and the result was averaged.

Time-dependent pH stability studies

A pH meter (Hana Instruments, Romania), was used to measure the formulations' pH over time for both drug-loaded and unloaded (drug-free) MPs after 24 h, 2, 4 and 8 weeks of storage condition (25 °C) in order to determine the formulations' stability with respect to product degradation during storage through standard procedure [9].

Determination of encapsulation efficiency (EE) and drug loading capacity (DLC)

Previously published techniques were used to assess the carriers' encapsulation efficiency (EE) and drug loading capacity (DLC) using Eq 1 and 2 with slight modifications [9]. The amount of insulin was measured using HPLC analysis [4]. An autosampler (AS-206, Japan), a UV–Vis detector (UV-2075, Jasco, Japan), a pump (PU-2089, Jasco, Japan), and a column C18 (250 mm x 4.6 mm; particle diameter 5 µm; Shimadzu, Japan) made up the chromatographic configuration.

Acetonitrile and phosphate buffer mixture (70:30, pH 7.4) was used as the mobile phase, and the flow rate was set at 1 mL/min. A 0.2 µm cellulose acetate membrane filter (Advantec, Japan) was used to filter the mobile phase. The entire run duration was 8 minutes, and the injection volume was 20 µL. The column temperature was kept at 25 °C, and the wavelength was 227 nm.

EE (%) = {(TI-IS)/TI}100 ……..…… (1)

DLC (%) = (Wi/Wp)100 …… (2)

where TI is the total amount of insulin added, IS is the insulin in the supernatant, Wi is the weight of encapsulated insulin and Wp is the total weight of the polymer.

Table 1: Composition of the different batches of insulin-loaded microparticles

Batch	Eudragit® RL100: P90G	Humulin 70/30 (mL)	PVA 2%w/v (mL)	P407 2%w/v (mL)	PEG 4000 (5, 10 or 20%) (10 mL)	Chitosan (2, 4 or 6%) (10 mL)
P1	9: 1	1.0	5.0	5.0	5.0	_
P2	9: 1	1.0	5.0	5.0	10.0	_
P3	9: 1	1.0	5.0	5.0	20.0	_
Q1	9: 1	1.0	5.0	5.0	_	2.0
Q2	9: 1	1.0	5.0	5.0	_	4.0
Q3	9: 1	1.0	5.0	5.0	_	6.0
R1	9: 1	1.0	5.0	5.0	5.0	2.0
R2	9: 1	1.0	5.0	5.0	10.0	2.0
R3	9: 1	1.0	5.0	5.0	20.0	2.0

***In vitro* drug release studies**

Insulin release from the MPs was studied using 100 mL phosphate buffer of pH 7.4 as a release medium at 37 ± 0.5 °C using magnetic stirrer (BioCote®, England) at 100 rpm. A 5 mL of each MP formulation was placed inside a dialysis membrane (MWCO: 10,000 – 16,000) tied at both ends and suspended in a 250 mL capacity beaker containing 100 mL of release medium.

The dialysis membrane was soaked overnight in distilled water before being used for the release study.

Using a UV spectrophotometer set to 271 nm, 5 mL samples of the release medium were taken out and replaced with an equivalent volume of the fresh medium at prearranged intervals to maintain sink condition [5].

Determination of *in vivo* oral hypoglycaemic effect of the insulin-MPs

In vivo studies using albino rats were performed according to the guide for the care and use of laboratory animals [10]. Permission was obtained from the Animal Ethics Committee of the University of Nigeria, Nsukka and the reference DOR/UNN/17/00014 was assigned. Rats were procured from the animal house of the Department of Pharmacology and Toxicology of the University of Nigeria, Nsukka. Five male rats each with an average weight of 107.5 g were used as positive control, negative control, and each of the test drug preparations. A single intra-peritoneal dose of aqueous solution of alloxan monohydrate 150 mg/kg of body weight was used to induce hyperglycaemia. Stable hyperglycaemia was confirmed on the 5th day and rats with persistent fasting blood glucose of greater than 150 mg/dL were considered diabetic and used.

Negative control group was administered 3 mL distilled water (vehicle). Positive control group received 50 IU/kg of regular insulin (Humulin®); while various test groups received doses of various batches of insulin-loaded MPs equivalent to 50 IU/kg body weight. Blood glucose levels of the rats were determined at different time intervals after dosing, by collecting blood samples from the caudal vein of the rats and testing them using Accu-check basic blood glucose monitoring system (Roche Diabetes Care, Inc.). Blood glucose levels at different time intervals after treatments were plotted against time [4,5].

Toxicological studies and biochemical evaluations

All treated groups received Humulin® 70/30 intraperitoneally or insulin-loaded MPs orally once daily for six days. Two hours after the last dose administration their biochemical and liver enzymes were evaluated. Analyses of samples for cholesterol (Tc), triacylglycerol (Tg), total protein (Tp), urea (U) and liver enzymes including alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were performed as described in the standard kits [4,11].

Statistical analysis

Data were analyzed using IBM® SPSS® Version 20.0 (New York, USA). All values were stated as mean ± standard deviation (SD). Differences between means were evaluated using one-way ANOVA or Student's t-test, and statistically significant differences were determined at $p < 0.05$.

RESULTS

Morphology and particle sizes of insulin-loaded microparticles

The particle sizes and morphology of the different batches of insulin-loaded microparticles varied depending on the polymer used. Some particles had perfect spherical shapes with smooth edges while others were non-spherical. The average size ranged from 2.14 ± 2.0 µm to 13.35 ± 13.3 µm (Table 2). The MPs made with PEG 4000 alone were observed to have smooth surfaces with obvious round appearances.

Encapsulation efficiency of insulin-MPs

Insulin-MPs had encapsulation efficiency of 62.55 to 94.50 % (Table 2). It was observed that over 60 % of insulin was incorporated in the microparticles, with batch R2 having highest encapsulation efficiency of 94.5 % and batch P3 having the lowest encapsulation efficiency of 62.55 %.

Table 2: Insulin-loaded MPs' particle size (PS), drug-loading capacity (DLC), and encapsulation efficiency (EE; n=5)

Sample	PS (µm)	EE (%)	DLC (%)
P1	3.18±2.2	79.46±0.22	28.75±0.11
P2	4.44±2.9	72.31±0.21	24.85±0.32
P3	7.34±0.4	62.55±0.32	19.55±0.43
Q1	2.14±2.0	84.93±0.02	31.72±0.32
Q2	6.72±1.5	88.68±0.11	32.42±0.42
Q3	8.41±4.1	88.70±0.41	31.75±0.22
R1	10.79±5.2	84.22±0.17	29.80±0.12
R2	13.35±0.3	94.50±0.72	31.84±0.62
R3	8.08±3.2	92.77±0.18	28.49±0.33

Time-dependent pH stability studies

All insulin-loaded MPs had pH values of 4.1 - 5.5 within the first 24 h after production (Table 3). All but two of the MPs experienced a slight increase in pH after two weeks of storage. Only Q3 retained the same pH after two weeks of storage

while a slight reduction in pH was noticed in R3 after the same period. Generally, there was no significant change in the pH of insulin-loaded MPs after storage for 8 weeks.

Table 3: Time-resolved pH analysis as a function of stability of the formulation

Batch	24 h	2 weeks	4 weeks	8 weeks
P1	5.5	5.9	5.9	5.9
P2	4.9	5.6	5.6	5.6
P3	4.6	5.5	5.4	5.5
Q1	4.1	4.2	4.3	4.3
Q2	4.1	4.3	4.3	4.2
Q3	5.0	5.0	5.2	5.3
R1	4.3	4.4	4.4	4.4
R2	4.2	4.3	4.4	4.4
R3	4.3	4.2	4.5	4.4

In vitro drug release

In vitro drug release studies showed that less than 50 % insulin release was achieved in the first 12 h, hence the formulation demonstrated a sustained release of the drug. There was no significant difference ($p < 0.05$) in the rate and the amount of insulin released by P1, P2 and P3 (Fig. 1A). Batches Q1 and Q2 made with 2 and 4 % CTS released 44.69 and 49.38 % of insulin, respectively. However, Q3 made with 6 % CTS achieved only 25.82 % drug release after 12 h (Figure 1B). Drug release from R1, R2 and R3 (made with a combination of PEG 4000 and 2 % CTS) was also retarded and the slowest of the three groups (Figure 1C). Only 30.4, 27.04 and 27.97 % were released from R1, R2 and R3, respectively after 12 h. The *in vitro* release of insulin from MPs seemed to be higher for P1, P2 and P3 than for the other batches containing CTS.

In vivo hypoglycaemic effect study

All the formulations showed a time-dependent decrease in blood glucose levels in the diabetic rats. However, a rise in glucose levels was noticed in some batches after the 6th hour. Among the insulin-loaded MPs made with PEG 4000 alone (P1, P2, and P3), P3 caused the greatest decrease in blood glucose level (17.4 mg/dL) followed by P1 (25.44 mg/dL), and then, P2 (27.2 mg/dL; Figure 2 A). The second group of insulin-loaded MPs made with CTS achieved even higher blood glucose reduction than all the other batches of MPs. Of the three batches in this group, Q1 and Q3 did exceptionally well (Figure 2 B). Glycaemic control by R1, R2, and R3 was not as pronounced as the previously discussed batches respectively (Figure 2 C). The animals in the control group that received water only experienced a sustained rise in blood glucose.

Toxicological and biochemical evaluation

Investigations carried out on the serum of treated diabetic rats as well as normal untreated non-diabetic rats used Tp, Tg, Tc, urea, SGPT, SGOT and ALP as biomarkers. A check on the concentration of these liver enzymes in the blood of animals treated with the MPs revealed amounts within acceptable reference ranges for all batches of MPs (Table 4). This was almost the case for the serum lipid profile, however, only the total protein and cholesterol were within acceptable reference ranges. The total triglyceride increased significantly with the induction of diabetes as seen in Figure 4. Interestingly, all the formulations of insulin-loaded MPs caused a significant reduction in Tg (Figure 3), with Q3 and R3 causing the highest reduction and resulting in the following Tg values: 208.16 and 210.41 mg/dL, respectively.

Table 4: Influence of the microparticles on the liver enzymes activities (IU/kg)

Group	SGPT	SGOT	ALP
DW	34.0±1.11	65.5±2.11	120.0±3.14
Insulin	36.0±2.13	61.5 ±2.41	122.0±1.21
Q1	36.0±1.33	67.0±1.12	110.0±2.12
Q2	39.0±1.02	67.0±1.41	115.0±1.21
Q3	37.0±0.33	67.5±1.21	114.0±1.21
P1	35.5±2.11	73.1±0.21	118.0±1.44
P2	36.0±1.14	69.5±1.21	117.0±1.22
P3	35.0±0.11	70.1±1.41	110.0±1.46
R1	33.5±1.11	69.0±1.44	118.0±0.21
R2	33.5±1.66	74.0±2.11	110.0±1.22
R3	34.5±1.45	75.0±0.11	116.0±2.11
Reference values	**10-40**	**50 - 150**	**30 – 13**

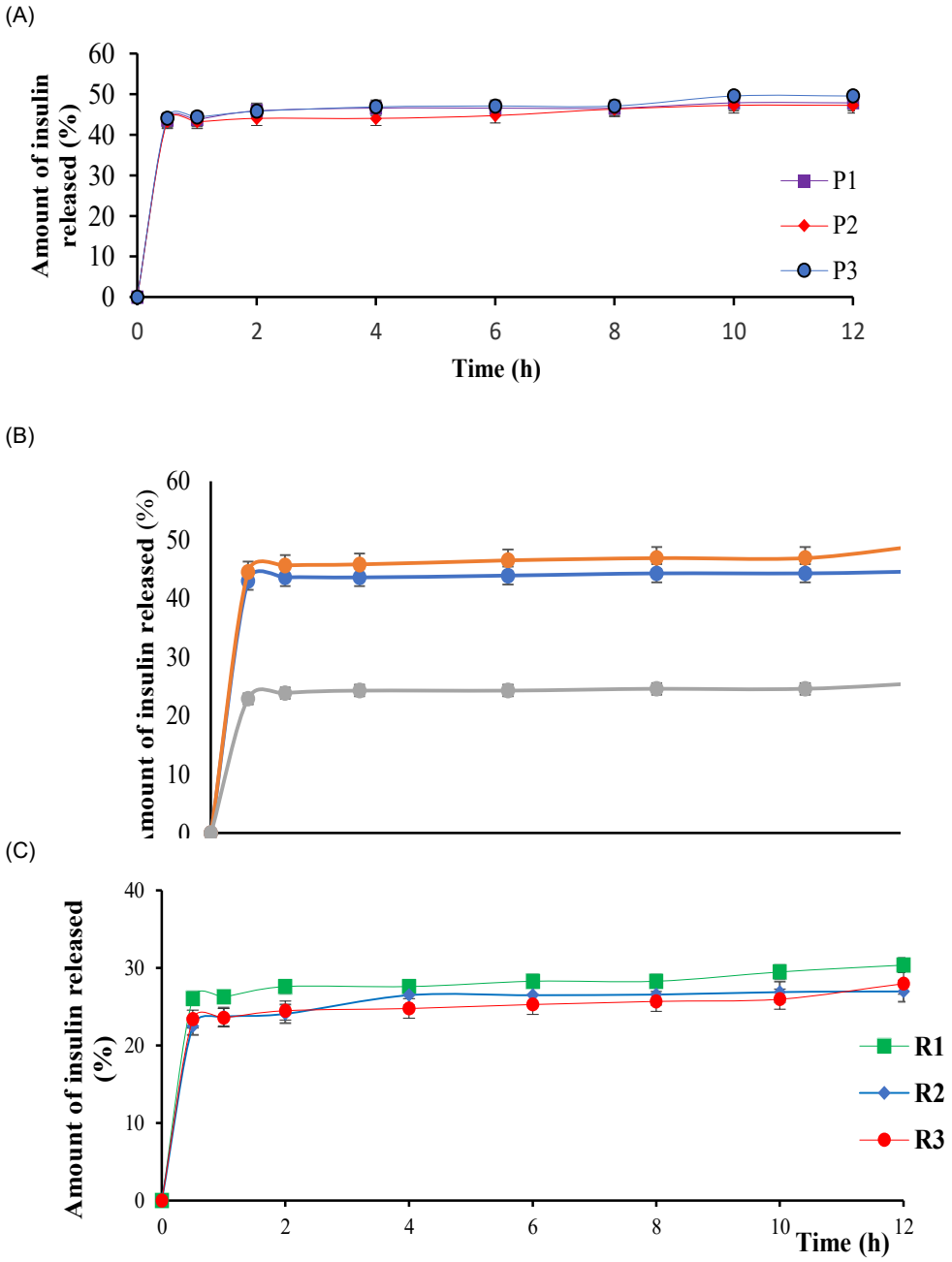

Figure 1: Release profiles of various batches P1, P3 and P3 (A); Q1, Q2 and Q3 (B); R1, R2, and R3 (C) in simulated intestinal fluid (pH 7.4)

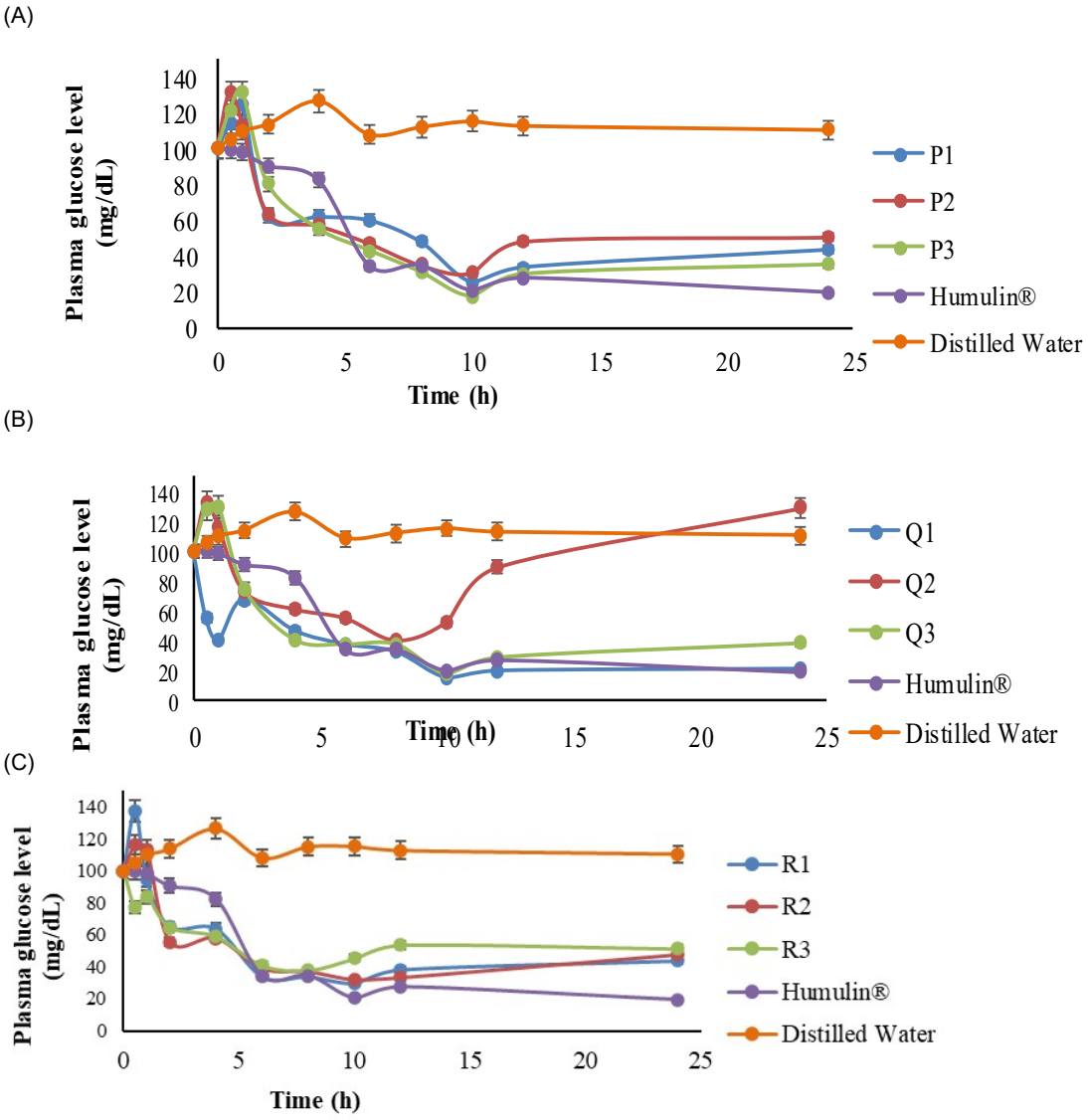

Figure 2: A presentation of plasma glucose level for P1, P2, and P3 (A); Q1, Q2, and Q3 (B); R1, R2, and R3 (C)

DISCUSSION

Nine batches of insulin-loaded MPs differentiated by the type of polymer and concentrations of polymer or polymer combinations were designed. For most batches of MPs, (excluding R1, R2 and R3) an increase in particle size was noticed with increasing concentration of polymer or polymer combination. In addition, an increase in particle sizes was observed with increasing concentration of polymer from 5 to 20 %, for PEG 4000 and from 2 to 6 %, for CTS.

This may be due to Ostwald ripening which may be attributed to the kinetic advantage of larger crystals when the concentration of the polymer is increased [4,12].

However, the batches made with a combination of PEG 4000 and 2 % CTS had particles that increased in size when the concentration of PEG 4000 increased from 5 to 10 % (R1 and R2), but smaller particles were produced when 20 % PEG 4000 (R3) was used. This may be attributed to the lubricant property of PEG 4000 which prevents individual particles from aggregating during and after formulation processes [13]. The clumped or aggregated particles noticed among CTS-containing MPs (Q1, Q2, Q3, R1, R2 and R3) may be caused by the concentration of CTS used.

An earlier study by Zhi *et al* reported the production of loosely formed insulin MPs, made with 10 % PEG and 1 % CTS [14]. This varies with the larger particle sizes observed with an

increase in PEG 4000 earlier reported [15]. The particle size and morphology which are controlled by the type and concentration of excipients and drugs used in the preparation of MPs are known to influence the formulation behaviour in terms of stability, solubility, release profile and kinetics, rate of absorption, permeability and bioavailability [12].

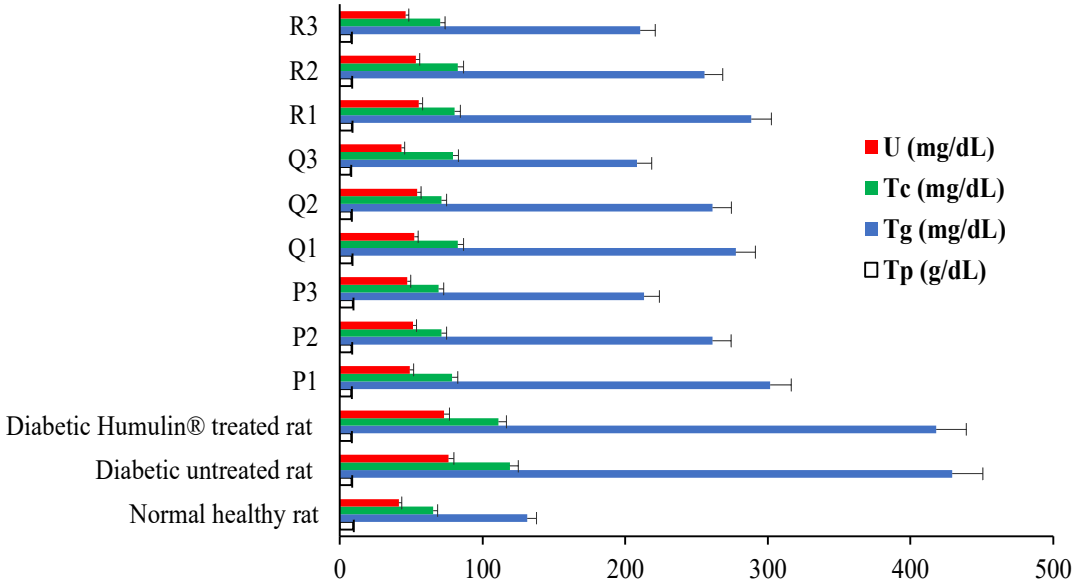

Figure 3: Total blood concentration of protein (Tp), triglycerides (Tg), cholesterol (Tc) and (U) urea in diabetic rats treated with insulin-loaded MPs

The encapsulation efficiency for PEG 4000 formulations decreased with increasing polymer concentration but increased with higher CTS concentration among the formulations containing CTS. EE increased when the concentration of PEG 4000 rose to 10 from 5 % among the batches prepared by combining both CTS and PEG 4000; however, a slight reduction in encapsulation efficiency was noticed when the concentration of PEG 4000 increased to 20 %. Hence, an increase in the concentration of PEG 4000 in this group of formulations did not increase the accommodation of insulin. The higher encapsulation efficiency of R2 compared to R3, despite the larger concentration of PEG 4000 may be caused by the larger particle size of R2 (13.35 ± 0.3 µm) than R3 (8.08 ± 3.2 µm). Insulin being a water-soluble hormone may have dissolved more in the aqueous region during formulation in the presence of high concentration of a water-soluble polymer such as PEG 4000, resulting in lower encapsulation efficiency. It was also observed that EE as well as DLC improved with increasing concentration of CTS in contrast to the drop in EE and DLC observed with increasing the concentration of PEG 4000. This may be attributed to the fact that chitosan being positively charged enhanced the encapsulation of the negatively charged insulin more than the other polymers.

Combining PEG 4000 and CTS resulted in higher encapsulation efficiency than when the polymers were used individually. This synergistic effect has earlier been reported by Zhi *et al,* [14]. The other reason for the high EE may be because insulin was not subjected to high speed and long homogenization, since insulin and other peptides tend to degrade when homogenized at high speeds for a long period [16,17].

All insulin-loaded MPs were mildly acidic with minimum and maximum pH values of 4.1 and 5.5 respectively within the first 24 h after production. All but two of the MPs experienced a slight increase in pH after two weeks of storage. Only Q3 retained the same pH after two weeks of storage while a slight reduction in pH was noticed in R3 after the same period. Generally, there was no significant change in the pH of insulin-loaded MPs after storage for 8 weeks. This is an indication that there was no degradation of the drug, polymer or other excipients used in the preparation.

In vitro drug release studies showed that less than 50 % insulin release was achieved in the first 12 h, hence the formulation demonstrated a sustained release of the drug. All batches of insulin-loaded MPs showed a biphasic release pattern which was characterized by an initial burst release of insulin within the first 30 min, followed by a very gradual and continuous release. There was no significant difference ($p < 0.05$) in the rate and the amount of insulin released by P1, P2 and P3 indicating that drug release was independent of PEG-4000 concentration. Increasing the concentration of CTS seemed to slow the rate and amount of insulin released among the batches made with CTS. Batches Q1 and Q2 made with 2 and 4 % CTS released 44.69 and 49.38 % of insulin, respectively. However, Q3 made with 6 % CTS achieved only 25.82 % drug release after 12 h. Drug release from R1, R2 and R3 was also retarded and dependent on the concentration of CTS.

The release of insulin (*in vitro*) from MPs was higher for P1, P2 and P3 than for the other batches containing CTS. PEGylation is known to improve the water solubility of proteins and this could be the factor behind the increase in drug release of the first batch formulated with PEG 4000 [18]. This result correlated well with the outcome of the release study (*in vitro*) where a higher amount of insulin was released from P3 than from P1 and P2. Interestingly, the reduction in blood glucose level caused by orally administered P3 at the 9th hour (17.4 mg/dL) was less than that caused by subcutaneously administered Humulin® even after 24 h (19.61 mg/dL).

The second group of insulin-loaded MPs made with CTS achieved even higher blood glucose reduction than all the other batches of MPs. Of the three batches in this group, Q1 and Q3 did exceptionally well. Q1 was even able to cause an immediate reduction in blood glucose level 30 min after administration, unlike all the other batches from the three groups, except R3. The other batches (P1, P2, P3, Q2, Q3, R1 and R2) exhibited an initial rise in glucose level upon administration, with reduction only starting an hour after the intake of insulin-loaded MPs. This initial increase in glucose level demonstrated by these batches may be attributed to the time taken for the MPs to travel across GIT as well as the time for the MPs to solubilize and release insulin.

Results of *in vivo* antidiabetic analysis showed that oral administration of insulin-loaded MPs had comparable effects on blood glucose levels as Humulin® (insulin) injection and so may serve as a more convenient alternative to insulin injection. A check on the concentration of these liver enzymes in the blood of animals treated with the MPs revealed amounts within acceptable reference ranges for all batches of MPs. The total triglyceride increased significantly with the induction of diabetes. Interestingly, all the formulations of insulin-loaded MPs caused a significant reduction in Tg, with Q3 and R3 causing the highest reduction and resulting in the following Tg values: 208.16 and 210.41 mg/dL, respectively. Overall, insulin-containing MPs were not hepatotoxic [19,20]. On the other hand, the feed given to the rats may have been highly proteinous resulting in high levels of blood protein and urea. However, orally administered MPs especially Q3 and R3 were able to cause a considerable reduction in these parameters better than Humulin® administered through the subcutaneous route.

CONCLUSION

Polymer-based insulin-loaded microparticles have been successfully formulated from blends of PEG-4000, chitosan and Eudragit®RL-100. These formulations showed characteristics in terms of average particle sizes, encapsulation efficiency and drug loading capacity. *In vitro* release profile indicates that drug release is highest for MPs prepared with PEG 4000 (P1, P2 and P3), while CTS-containing groups showed a more sustained release of insulin from MPs. *In vivo,* the antidiabetic analysis revealed that oral administration of all batches of insulin-loaded MPs had glycaemic control that is comparable to that achieved by Humulin® administered through the subcutaneous route. The results obtained reflect the potential of these polymer-based microparticles as convenient drug delivery systems for oral administration of insulin. Additionally, these MPs have high potential of being able to improve insulin sensitivity and prevent insulin resistance, as well as defective insulin release.

DECLARATIONS

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Ethical approval

The Animal Ethics Committee of the University of Nigeria, Nsukka approved the use of these animals for these experiments with a reference number DOR/UNN/17/00014.

Availability of data and materials

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

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Mumuni A. Momoh and Hanifah Abdulmumin conceptualized the research and designed the experiments. Chinazom P. Agbo, Josephat I. Ogbonna, Chinenye C. Chukwu, John Alfa, Nafiu Aminu, and James Oyeniyi performed the experiments and collected the data. Umar S. Okino, Franklin C. Kenechukwu, and Deghinmotei Alfred-Ugbenbo analyzed and interpreted the data.

Josephat I. Ogbonna and Mumuni A. Momoh wrote both the original draft and the final copy of the paper. Darlington C. Youngson contributed materials, tools, reagents, and supervised the research.

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