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Development and Characterization of Mucinated Chitosan Microcomposite for Oral Insulin Delivery

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ABSTRACT

The development of oral insulin is a vital tool in improving compliance in diabetic patients. The study aimed to develop oral insulin microparticles using mucin grafted chitosan as the carrier matrix and to study the *in vitro* and *in vivo* properties of the formulations. Insulin-loaded microparticles (IMP) were prepared by water-in-oil-in-water (w/o/w) double emulsion technique. Varying ratios of mucin to chitosan; 1:1 (FA1), 1:2 (FA2), 1:3 (FA3), and 1:4 (FA4) were used in the preparation of the microparticles. The loaded-microparticles were characterized *in vitro* by encapsulation efficiency (EE), particle size, morphology, and release studies. The *in vivo* hypoglycaemic effects of the IMP were studied in alloxan-induced diabetic rats. The results showed a recovery value of ≥ 73%, particles sizes ranging from 121.0 ± 0.04 µm to 142.6 ± 0.05 µm. EE% ranged from 91.1% to 93.7%. The *in vitro* release of insulin from the microparticles at 8 h were 30.5, 37.8, 49.0, and 58.2% for FA1-FA4, respectively. The *in vivo* hypoglycemic studies showed that orally administered IMPs significantly ($p < 0.05$) lowered the blood glucose and showed glucose reduction from 89.23 ± 1.72% at 0.5 h to 26.46 ± 4.02% in 12 h. Liver function tests showed no significant changes compared with the control. The results suggest that mucin grafted chitosan microparticles possess good potentials for oral delivery of insulin and offered sustained drug release effects which could be useful in the oral delivery of insulin.

Keywords: Mucin, Chitosan, Oral insulin, Microparticles.

Introduction

Natural polysaccharides such as chitosan offer several advantages for the replacement of synthetic polymers in pharmaceutical and biomedical applications due to their low cost, non-toxicity, biodegradability, and availability.[1] Chitosan, which is obtained by partial or total deacetylation of chitin, is a promising delivery agent used in pharmaceutical and biomedical applications.[2] The increasing interest in its use in pharmaceutical industries is as a result of its biocompatibility, nontoxicity, abundance, mucoadhesive as well as the fact that it is biodegradable.[3] Chitosan has been investigated in various particulate drug delivery systems.[4-6] Research has shown that chitosan in oral delivery enhanced oral absorption of drugs by providing electrostatic interaction with negatively charged mucous content of the gastrointestinal tract system.[4-6] On the other hand, snail mucins are glycoproteins with mucoadhesive properties and are ubiquitous in many human and animal tissues. They are negatively charged, hence can serve as a good candidate for drug delivery as they can be conjugated to positively charged drug directly or polymer such as chitosan to create a new entity of polymer matrix with superior properties as compared to individual polymer, with the sole aim of effective delivery of drug to target.[7] Insulin, an anti-diabetic drug that has been the mainstream in the treatment of diabetic patients is usually administered by subcutaneous injection. This route of drug administration suffers from patient noncompliance due to associated pain, among others side effects. Hence, oral delivery of insulin has become a solution to overcome the problem of patient non-compliance leading to better overall therapeutic outcome and better disease management. Interestingly, orally administered insulin simulates the normal physiological pathways by directly delivering insulin into the liver after absorption.[8-11] Numerous chemically synthetic-based polymeric drug delivery systems have been developed viz: microsphere, micelles, hydrogel, nanoparticles to facilitate the oral delivery of protein and peptide.[12] However, the production cost of synthetic polymers is not easily affordable and the unhealthy interaction between the chemical and the peptide material has been widely reported.[11] More so, an ideal delivery system for oral insulin, should be enzymatically stable in the gastrointestinal system, possess high drug encapsulation, improve mucosa adhesion to the gastrointestinal wall, and easily diffuse through the mucus membrane for the effective therapeutic purpose.[13] Chitosan has had wide pharmaceutical applications owing to its high mucoadhesion and positive surface charge. Research has shown that chitosan can enhance protein stability, increase its duration of action in the systemic circulation, and allow for drug administration through the oral route.[1] However, the ease of protonation has been its major challenge as this brings about the premature release of the drug before the absorption site. It is hypothesised that by hybridization of chitosan with acetone-based extract of snail mucin in the form of microparticles, utilising the individual advantage and carefully avoid their disadvantages, the two bioresource materials could form a novel polymer microparticle for effective oral delivery of insulin. The study, therefore, aimed to design a novel natural bioresource polymer entity prepared as microparticles based on

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electrostatic interactions between the two polymers and also evaluate its pharmaceutical application in the delivery of oral insulin. The *in vivo* effect of the formulation was evaluated in alloxan-induced diabetic rats.

Materials and Methods

The following chemicals were bought from their suppliers and used without further purification: Acetone (Merck, Germany), High molecular weight chitosan (Sigma USA), Span 60, Humulin 70/30 (Eli lily and company, USA), ethanol, paraffin oil (Moko pharm., Ltd Lagos, Nigeria), Terrestrial African giant snails were purchased from the Ogige local market in Nsukka, Enugu state. All other chemicals used were reagent grade.

Extraction of snail mucin

Snail mucin was extracted from the African giant snail *Archachatina marginata* following the method of Adikwu[7] and Arhewoh *et al*.[14] The snail shells were cracked and their fleshy bodies removed from the shells with the aid of a metal rod. Excretory materials accompanying the bodies were removed. The snail bodies were subjected to washing by gently squeezing off the slime from the fleshy bodies repeatedly into a pool of 250 mL of water and decanted. This procedure was repeated several times to obtain a pool of mucous from the snails. Mucin was precipitated with chilled acetone, filtered, and lyophilized to obtain brownish flakes. Finally, the dried flakes were blended in an electric blender to give mucin powders and were stored in an airtight container for further study.

Formulation of microparticles

The microparticles were formulated by a combination of two polymers (polycation chitosan and a polyanion snail mucin) using w/o/w double emulsion method. The organic phase was first prepared by dissolving 1 g of chitosan in 100 mL of glacial acetic acid to produce a 1% v/v solution. The chitosan solution was sonicated at an amplitude of 80 rps for 60 s using an ultrasonic probe (Athena Technology Virginia, USA). The aqueous phase was prepared using 1 g of snail mucin, dispersed in 20 mL of distilled water using a 100 mL beaker and the solution was sonicated at an amplitude of 80 rps for 60 s. About 10 mL of 100 I.U/mL of insulin (Humulin 70/30) solution was added dropwise to the mucin dispersion and was gently mixed using a magnetic stirrer (Remi Equipment Pvt Ltd, Mumbai). A 5 mL of 0.5% span 60 was added to a mixture of ethanol and liquid paraffin in the ratio of 1:1 in a 100 mL beaker and properly stirred using a stirrer. The aqueous phase containing insulin and mucin dispersion was gradually added dropwise using a syringe into the beaker containing the liquid paraffin mixtures and was kept under magnetic stirring at 100 rpm. To the above solution, the organic phase was gradually added and allowed for continuous stirring for 3 min. Thereafter, the emulsification was further subjected to gentle sonication at 20 rps for 20 s. The prepared microparticles were divided into two portions, one portion was lyophilized and the other part was stored without lyophilisation at a temperature of 20^{o}C for further use. The procedure was repeated using a mucin-chitosan ratio of 1:1, 1:2, 1:3, and 1:4 to obtain different batches of microparticle (FA1, FA2, FA3, and FA4, respectively).

Determination of percentage recovery values

The moisture-free microparticles from each batch were weighed after lyophilisation to get the microparticle values. The percentage yield was calculated using the formula:

$$\text{Production yield (\%)} = \frac{W1}{W2+W3} \times 100$$

Where, W1 = weight of microparticles recovered (g), W2 = weight of drug, W3 = weight of polymers + other excipients.

Particle size analysis

The particle size analysis was carried out on all the formulation batches 24 h after the formulation using a digital light microscope (Leica Germany) and an image captured with Moticam 1000 camera, China (magnification ×40). The morphology (shape and surface) of the particles was evaluated. All measurements were done in triplicate. Also, the samples were diluted with 10 mL of double-distilled water to obtain the required scattering intensity, before photon correlation spectroscopic analysis.

pH analysis

With the aid of a pH meter (Horiba pH meter Japan), the pH of the various batches of the insulin-loaded microparticle, including those of the control was measured. This was carried out at different time intervals (day 1 to 3 months).

Encapsulation efficiency (EE%) and Drug loading capacity (DLC)

The EE was evaluated using 50 mg of IMP added into a micro concentrator (Vivaspin 6, Vivascience Honover, Germany) and 5 mL of phosphate buffer was added to each. The dispersion was centrifuged at 3000 rpm for 60 min. The supernatant was filtered and analyzed using UV/Vis spectrophotometer at 271 nm (UNICO 2102 PC UV/Vis Spectrophotometer, USA). The amount of insulin encapsulated (EE) and the drug loading (DLC) in the microparticles was calculated using the following the formulae:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Weight of drug assayed in MP}}{\text{Weight of drug fed initially}} \times 100$$

$$\text{DLC (\%)} = \frac{\text{Weight of drug in microparticles}}{\text{total weight of microparticles}} \times 100$$

In vitro drug release study

In vitro drug release study of the microparticles was evaluated in freshly prepared phosphate buffer solution (pH 7.2) using a dialysis membrane technique. The polycarbonate dialysis membrane (MWCO 8000–10,000 Spectrum Labs, Netherlands) used as a release barrier was soaked in a phosphate buffer solution for 24 h before its use in the study. Insulin loaded microparticles equivalent to 50 mg was placed in a dialysis membrane containing 5 mL of the dissolution media was secured at both edge with thread and was suspended in a 200 mL phosphate buffer in 500 mL-beaker at pH 1.2, agitation was provided by magnetic stirrer at speed of 100 rpm for a period of 3 h, thereafter the dissolution medium was changed to pH of 7.2, in all case, the experiment were maintained at 37.0 ± 0.5°C. At predetermine interval of time, 5.0 mL sample of dissolution medium was withdrawn, filtered through a 0.22 µm filter (Millipore®, USA) and assayed using a UV-spectrophotometer at 271 nm to determine the drug absorbance. At every withdrawer made, equal amount of the same medium were added to maintain a sinking condition. The above procedure was repeated using the remaining batches.

Antidiabetic studies

Adult Wistar rats weighing 150 – 170 g were purchased from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and were maintained at standard housing conditions (room temperature, 25^{o}C) with 12 h light. The animals were allowed to acclimatize for 7 days, during which they were fed with a commercial diet (Feeds BC, Nsukka, Nigeria) and water. All animal experimental procedure followed the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, and the European Community guidelines (EEC Directive of 1986; 86/609/EEC).
Alloxan monohydrate solution (Sigma, USA) was freshly prepared before injection. A stock solution of alloxan monohydrate was prepared by dissolving 10 g of alloxan to 100 mL of normal saline (0.9% w/v NaCl) to obtain a concentration of 100 mg/mL. The rats were rendered diabetic before the study by intraperitoneal injection of 1 mL of the stock solution of alloxan. Thereafter, the blood glucose levels were regularly monitored at various intervals; four times daily for three days using glucometer (ACCU-CHECK, Roche, USA). Food

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intake was measured in (g), water (mL), and urine volume (mL) daily. The rats were confirmed diabetic 5-7 days after the administration of solution of alloxan when the glucose levels were in the range of 220 – 250 mg/dL. The diabetic rats were randomly divided into four groups of seven rats per group. All animals were fasted for 12 h before the experiment but had free access to water throughout the study. The rats in group one (positive control) received 2.5 IU/kg insulin subcutaneously, group two (negative control) were given 25 IU/kg of plain insulin through the oral cavity, group three (Test samples) were given 25 IU/kg of insulin loaded microparticles orally using a nasogastric tube, and group four received 25 IU/kg of unloaded formulations. The blood glucose was determined using Accu check glucose meter and blood glucose test strip at predetermined time intervals of 0, 0.5, 1, 3, 6, 9, 12, and 24 h and was expressed as a percentage of the baseline plasma glucose level.

Toxicological evaluations

The liver function tests were investigated as a function of toxicological study. Herein, 15 rats were purchased and acclimatized in the Animal House as stated in the antidiabetic evaluation section. The rats were made diabetics following the procedure used in an *in vivo* evaluation as stated earlier. Thereafter, the rats were fractioned into three groups of 5 rats per group and were dosed as follows:

- Rats in group I received water 5 mL (served as a control),
- Rats in group II were treated with batch FA1 at a dose of 50 IU/kg, orally.
- Rats in group III was treated with batch FA2 at a dose of 50 IU/kg, orally.

All administration of the formulated Insulin-loaded microparticle was done orally once daily for three days. Blood samples were collected six hours after the last dose in three days as previously described in anti-diabetic section above. The samples were analysed for changes in biochemical parameters such as alkaline phosphatase (ALP), aspartate aminotransferase or serum glutamic oxaloacetic transaminase (AST or SGOT), and alanine aminotransferase or serum glutamic pyruvic transaminase (ALT or SGPT) using an automated Reflotron-plus machine (model, SN74746). All the tests were done in triplicates and results were averaged.

Statistical analysis

All experiments were repeated at least three times, Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan post hoc test and Dunnett multi comparison test using the INSTAT 2.00 Macintosh software (San Diego, CA). The difference was considered significant when $p < 0.05$.

Results and Discussion

Particle size and morphology

The particle size of the insulin-loaded mucin grafted chitosan microparticles is shown in Table 1. The results show that particle size ranged from 121.0 ± 0.04 to 142.6 ± 0.20 µm in all the formulation (FA1 to FA4). The photomicrographs shown in Figure 2 revealed that the microparticles have a spherical shape with a smooth surface and all the particle sizes were within the acceptable range that would facilitate oral absorption of insulin. Results also revealed that an increase in chitosan concentration insignificantly decreased the particle size ($p > 0.05$) of formulations. The result showed that an increase in the concentration of polymers resulted in a decrease in particle size. However, the particle characteristics of a formulation are necessary to ensure the production of stable products of suitable quality since physical stability and cellular uptake of particles are affected by particle size.[15] Size distribution is affected by production temperature, rate of stirring, the quantity of polymer, and the viscosity of the continuous phase.[15]

Yield, Encapsulation efficiency, and loading capacity

The results of the percentage yield of microparticles are shown in Table 1. The recovery rate was generally high and fall within the range of 77 to 82%. Encapsulation efficiency (EE) ranged from 91 to 93% and was not significantly affected by polymer concentration ($p < 0.05$) as shown in Table 1. However, the EE was generally high, indicating that the insulin was entrapped well in the formulations and attesting to the suitability of procedures used in the formulation. Furthermore, this observation could be attributed to the electrostatic interaction between positively charged chitosan and negatively charged snail mucin coupled with the viscoelasticity of mucin matrix thereby leading to high entrapment of insulin and may lead to prolonged release of insulin from the formulation. The determination of drug incorporation helps to evaluate a potential carrier system for control drug release. It is desirable to produce microparticles with high drug content to decrease the amount and frequency of the microparticles to be administered. All the formulations showed a high encapsulation efficiency of insulin an indication that the interaction between snail mucin and chitosan resulted in firm entrapment of the drug into the polymer core. However, the encapsulation efficiency decreased with an increase in the concentration of chitosan in the formulation which may be possibly due to a decrease in the viscoelasticity property of mucin with an increase in chitosan ratio. The LC (Table 1) showed a range of 0.7 to 1.7 which also showed good results confirming that the matrix carrier chosen was good enough.

pH analysis

The results of the pH stability study of various batches of the formulations are shown in Table 2. The results showed good stability of the formulation over the study period as depicted by insignificant ($p < 0.05$) variation in the overall pH over time. On day 1, after the production, the pH of insulin in the microparticle dispersion was found to be within 3.7 to 4.9, lower than the isoelectric point (the pH at which the net charge on the protein is zero) of insulin which is 5.3.[16,17] The pH of insulin in the dispersions remained relatively below its isoelectric point throughout the three months tested. This shows that the interaction between positively charged chitosan and negatively charged snail mucin resulted in the stabilization of insulin in the formulation.

In vitro drug release study

The release profile of insulin loaded microparticles formulated using SMC is shown in Figures 2a and 2b. The release of insulin in an acidic medium showed a maximum release of 8% within the first three hours of the evaluation. This indicates that the formulation was able to prevent any significant release from the formulation. The recorded value could be attributed to the unencapsulated insulin in the surface of the microparticle. On the other hand, the release of insulin in the pH 7.2 were higher and maintain a consistent release from the microparticles throughout the 8 h duration of the study, with highest release of 58.5% for batch FA4. This pattern of release was observed when chitosan and Arabic gum were used for similar evaluation.[18] This may be possibly due to the electrostatic interaction between negatively charged component of the sialic acid content of snail mucin and positively charged surface of chitosan at a pH of ≤ 6.5 thereby limiting insulin release into the dissolution medium.[19] Additionally, prevention of the protonation of the chitosan as a result of the activities of mucin forming a gel network and decrease the swelling tendency of microparticle, all these are pointer toward poor insulin release in acidic medium and also the gradual release observed at pH of 7.2.[20] In another words, the gradual and prolonged release of insulin from the microparticles could also be attributed to the role of the mucin network in retardation of drug expulsion due to its high viscoelasticity and consistency.[7] The percentage of insulin released from the formulation at 8 h was 30.5%, 41.5%, 49.0%, and 58.5% for FA1 – FA4, respectively prepared with SMC 1:1, 1:2, 1:3, and 1:4, an indication that most of the batches did not release 100% of their drug content and thus did not reach their maximum release in the time of the study.

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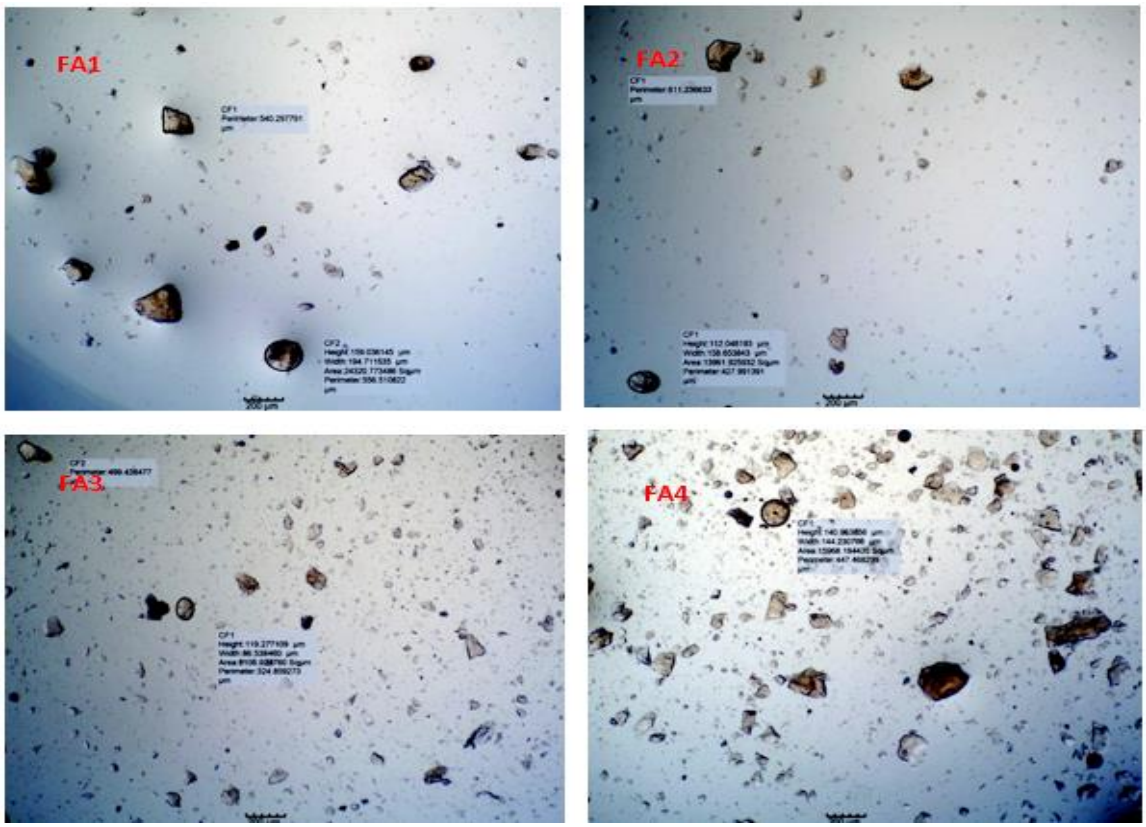

Figure 1: Photomicrograph of insulin-loaded microparticles prepared with SM: C; Batch FA1 contain 10 mL of insulin in 1:1 SM: C, Batch FA2 (10 mL of insulin in 1:2 SM: C), Batch FA3 (10 mL of insulin in 1:3 SM:C), Batch FA4 batch (10 mL of insulin in 1:4 SM:C); SM:C - snail mucin and chitosan.

Table 1: Some physicochemical properties of insulin-loaded microparticles

Batch	Loading capacity (%)	Encapsulation efficiency (%) (n = 3)	Production yield (%)	Particle size (µm ± SD)
FAI	1.7	93.7 ± 1.90	77 ± 0.01	140.4 ± 0.10
FA2	1.2	92.3 ± 1.70	79 ± 0.00	133.4 ± 0.05
FA3	0.9	91.1 ± 1.10	82 ± 0.10	121.0 ± 0.04
FA4	0.7	92.2 ± 1.80	76 ± 0.20	142.6 ± 0.20

Batch FA1 contain 10 mL of insulin in 1:1 SM:C, Batch FA2 (10 mL of insulin in 1:2 SM:C), Batch FA3 (10 mL of insulin in 1:3 SM:C), Batch FA4 batch (10 mL of insulin in 1: 4 SM:C); SM:C - snail mucin and chitosan.

In vivo antidiabetic activity of the formulations

Changes in blood glucose levels versus time after oral administration of unloaded microparticles, insulin-loaded FA1, FA2, insulin solution, and subcutaneous administration of insulin to overnight fasted diabetic rats are shown in Figure 3. The mean blood glucose baseline value was taken as a 100% level. After subcutaneous administration of insulin solution (2.5 IU/kg), the blood glucose level decreased to 50.57 ± 8.14% after 0.5 h, to a maximum decrease of 33.15 ± 7.01% after 1 h and then increased gradually. Administration of pure solution of insulin via oral route at a dose of 25 IU/kg only resulted in a maximum decrease of 86.71 ± 4.41% and 86.59 ± 7.13% at 1 h and 6 h, respectively. This is expected since a small fraction of insulin could be absorbed through the intestinal wall before degradation by the proteolytic enzymes. After oral administration of insulin loaded batch FA1 at a dose of 25 IU/kg, a gradual decrease in blood glucose level was observed from 89.23 ± 1.72% in 0.5 h to a maximum of 26.46 ± 4.02% in 12 h before it gradually increased to 34.39 ± 4.27% at 24 h duration tested. For the insulin loaded batch FA2 administered at the same dose range, a similar decline in blood glucose level from 102.87 ± 8.50% after 0.5 h to a maximum of 44.48 ± 7.57% in 12 h was noticed as seen in Figure 3. The absence of a hypoglycaemic effect after oral administration of unloaded microparticles (FA0) is an indication that the hypoglycaemic effect observed was solely due to the pharmacological effect of insulin.[17] At 3 h to 24 h post-administration of insulin-loaded microparticles (FA1, and FA2) to alloxan-induced diabetic rats, there were significant differences ($p < 0.05$) in the percentage blood glucose reduction compared with the standard pure insulin sample. This was because the percentage reduction in blood glucose level in animals that received the formulation (FA1, and FA2) was higher than the unprotected insulin solution that served as negative control. The result clearly indicates that the polymer based microparticle was able to protect the incorporated insulin against degradation by proteolytic enzymes in the gastrointestinal tract. This observation was in agreement with earlier reports.[20, 21]

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The insulin-loaded microparticles (FA1, and FA2) also showed a significant difference ($p < 0.05$) in blood glucose reduction at 12 h and 24 h compared to the subcutaneous injection, this is because the formulation showed a higher ability to decrease blood glucose concentration than the standard subcutaneous injection. Insulin subcutaneous injection (positive control) showed a faster percentage decrease in initial glycemic level than the insulin-loaded microparticles up to 6 h, indicating a better hypoglycaemic property compared to the microparticles, which is in line with previous reports. However, the microparticles had a gradual and prolonged blood glucose reduction effect than the subcutaneous injection. These prolonged and gradual blood glucose reduction effect observed with the insulin-loaded microparticles (FA1 and FA2) suggests that mucin grafted chitosan microparticles were able to stabilize insulin from degradation in the harsh conditions of the stomach, thereby allowing a significant fraction of intact insulin to reach and adhere to the site of absorption, and hence enter the systemic circulation via the paracellular pathway.[21-23]

Toxicological study

The results of the toxicological study of insulin loaded microparticles are shown in Table 3 and showed that the SGPT, SGOT, and ALP in the serum of animals treated with the formulations (FA1 and FA2) ranged from 38.0 ± 0.10 – 39.5 ± 0.11 IU/L, 66.0 ± 0.23 – 65.0 ± 0.31 IU/L, and 113.0 ± 0.13 – 112.0 ± 0.22 IU/L, respectively. However, the control had 36.0 ± 0.11, 64.0 ± 0.12, and 115.0 ± 0.30 IU/L for SGPT, SGOT, and ALP, respectively as shown in Table 6 below. This indicated that the mucin grafted chitosan insulin-loaded microparticles are safe with no hepatotoxic effect since the levels of the investigated enzymes were within normal range and no significant variation with the control.

Table 2: pH-dependent stability study of insulin loaded microparticles (n = 3)

Batch	Day 1	Day 14	Day 30	Day 60	Day 90
FA0	3.7 ± 0.00	3.8 ± 0.06	3.7 ± 0.10	3.8 ± 0.06	3.8 ± 0.10
FA1	3.6 ± 0.10	3.4 ± 0.00	3.2 ± 0.10	3.3 ± 0.20	3.4 ± 0.00
FA2	3.8 ± 0.10	3.5 ± 0.10	3.3 ± 0.00	3.3 ± 0.10	3.6 ± 0.06
FA3	3.8 ± 0.00	3.6 ± 0.10	3.3 ± 0.10	3.4 ± 0.06	3.6 ± 0.06
FA4	3.8 ± 0.20	3.5 ± 0.00	3.2 ± 0.06	3.5 ± 0.00	3.6 ± 0.00

Batch FA1 contain 10 mL of insulin in 1:1 SM:C, Batch FA2 (10 mL of insulin in 1:2 SM:C), Batch FA3 (10 mL of insulin in 1:3 SM:C), Batch FA4 batch (10 mL of insulin in 1:4 SM:C); FA0: Bland formulation; SM:C - snail mucin and chitosan.

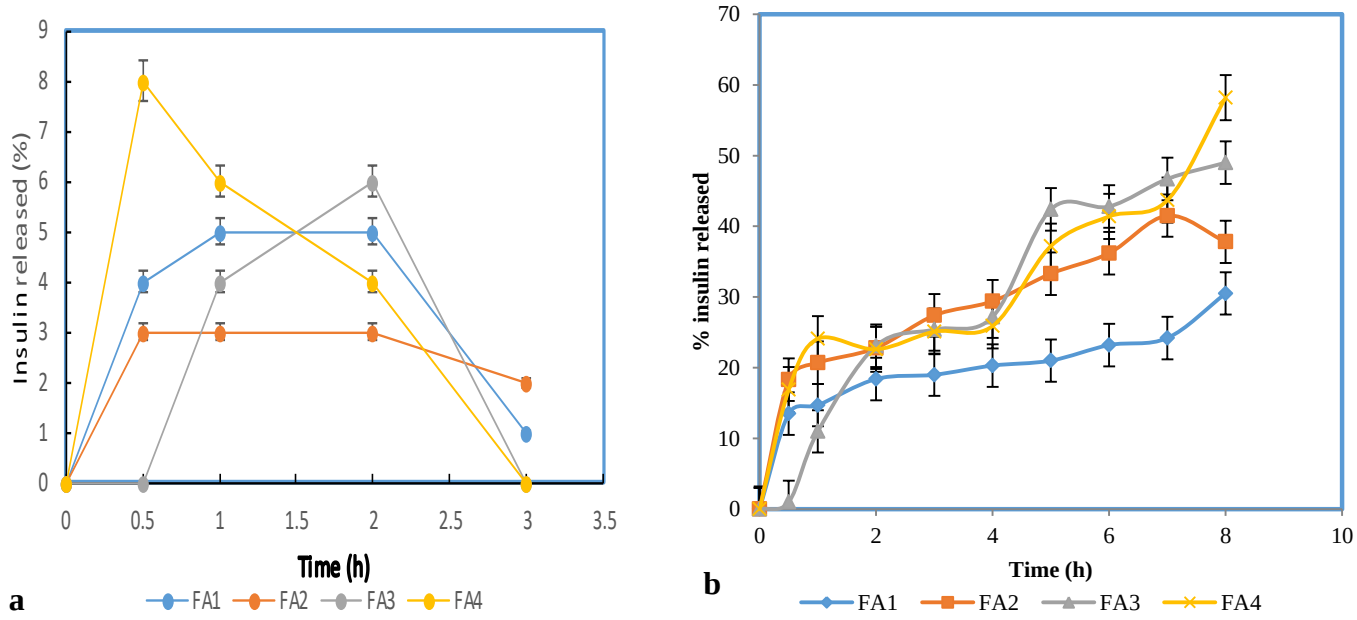

Figure 2: Release profiles of insulin from insulin-loaded microparticles (systems A1-A4) in **(a)** in acidic pH 1.2 and **(b)** in phosphate buffer pH 7.2, Abbreviation: FA1 = contain 10 mL of insulin in 1:1 SM:C: FA2 = (10 mL of insulin in 1:2 SM:C); FA3 = (10 mL of insulin in 1:3 SM:C); FA4 = (10 mL of insulin in 1:4 SM:C). Note; SM = snail mucin, C = chitosan. Data are presented as the mean ± standard deviation (n = 3).

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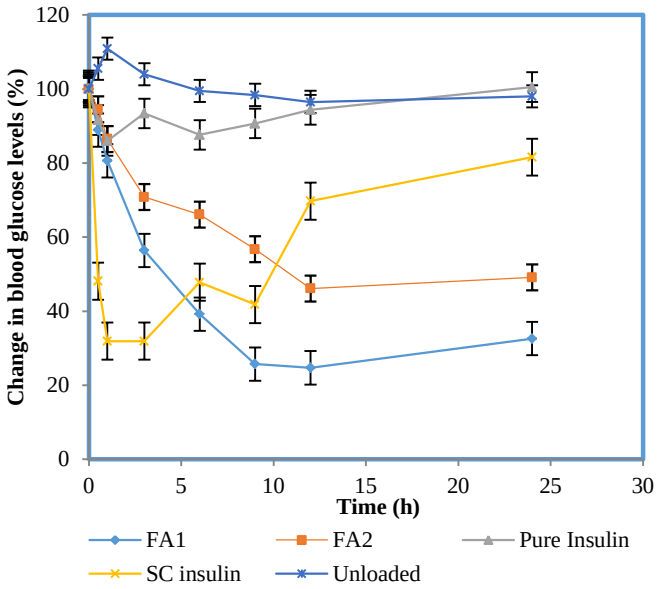

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Figure 3: Change in blood glucose level after a single oral administration of insulin solution (25 IU/kg), insulin-loaded microparticles (FA1 and FA2) at (25 IU/kg), unloaded microparticles, and SC injection of insulin (2.5 IU/kg).
FA1 = contain 10 mL of insulin in 1:1 SM:C; FA2 = contain (10 mL of insulin in 1:2 SM:C). Note: SM = snail mucin; C = chitosan; SC = subcutaneous. Data are presented as the mean ± standard deviation (n = 7).

Table 3: Toxicological studies of insulin-loaded mucin grafted chitosan microparticles

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Control	36.0 ± 0.11	64.0 ± 0.12	115.0 ± 0.30
FA1	38.0 ± 0.10	66.0 ± 0.23	113.0 ± 0.13
FA2	39.5 ± 0.11	65.0 ± 0.31	112.0 ± 0.22

Abbreviations: Alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), alanine aminotransferase or serum glutamic pyruvic transaminase (SGPT); FA1 = contain 10 mL of insulin in 1:1 SM:C: FA2 = (10 mL of insulin in 1:2 SM:C). Note; SM = snail mucin, C = chitosan. Data are presented as the mean ± standard deviation.

Conclusion

The insulin-loaded mucinated chitosan microparticles were stable, possessed good physicochemical properties, with better and sustained hypoglycaemic effect than pure oral insulin without carrier, however, the subcutaneous insulin injection slightly shows a higher effect than the test formulation at initial, but could not sustain the effect as to that of the polymeric formulation. This study has shown that insulin-loaded microparticles formulated using chitosan and snail mucin as the carrier could be further employed to enhance oral delivery of insulin for effective treatment of insulin-dependent diabetes, thus necessitating further investigations and scale-up.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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