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# Surface-modified mucoadhesive microparticles as a controlled release system for oral delivery of insulin

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ABSTRACT

To overcome barriers and improve oral bioavailability of insulin delivery has been a mirage to formulation scientists due to instability of the insulin after oral administration. Microparticle (MP) composed of chitosan and snail mucin was prepared via double emulsion method for oral delivery of insulin. Microparticles were characterized by differential scanning calorimetry, Fourier transform infrared spectroscopy and scanning electron microscopy. The encapsulation efficiency (EE) of the insulin-loaded MPs were evaluated. Insulin release behavior was evaluated in acidic and phosphate buffer (pH 1.2 and 7.4) at 37 °C. Bioactivities of insulin-loaded MPs were evaluated in a diabetic animal model after oral administration. The insulin-loaded MPs showed irregular shape with a zeta potential (>29 mV). The encapsulation efficiency and drug loading were >75 and 28 %, respectively. The *in vitro* release shows >80 % release of insulin over 12 h in a sustained manner. The insulin-MPs significantly reduced blood glucose levels (>50 %) compared to positive control and the effect lasted for over 8 h. This study suggests that insulin-MPs as prepared would be potential carriers for oral delivery of insulin.

## 1. Introduction

In recent years, there has been a dramatic increase in the rate of which hybridization of polymer for effective drug delivery has become the frontier of research rather than seeking for newer drug molecule due to the task involved [1]. This is so because the procedure for new drug molecule is very tedious, time-consuming and in most cases the researches never go beyond laboratory bench because of the inability of the study to produce the expected clinical result that could of market value.

Thus, the desire for combination of polymer through a process of hybridization or PEGylation to create a new polymer entities with improve/superior properties and avoid the disadvantage of individual polymer become necessary. The new polymer entities are to effectively deliver the drug molecule, while by-passing the short-falls associated with conventional drug delivery system. In addition, the new polymer entities are recognized to perform better as to generally achieve wide applications: targeting, protection, modification of circulation time, and better bioavailability among other advantages [1].

Chitosan (CS) is a natural polycationic and copolymer composed of deacetylated glucosamine and N-acetyl-D-glucosamine, which are primary building blocks for osteoarthritis treatments [2].

CS is an important natural polymer that has gained attention in the field of drug delivery, food processing, cosmetics and tissue engineering application because of its good biocompatibility, biodegradability and bioactivity [3, 4]. CS has been proved to have modified the permeability of the intestinal mucosa to peptides drug such as insulin by opening the tight junctions between epithelial cells thereby allowed the paracellular uptake of insulin [5, 7].

Mucin obtained from African giant snail has form the frontier in drug delivery as a result of it mucoaldhesive properties. For instance; bovine mucin alone or in combination with PEG-4000 has been reported to improve the delivery of antidiabetic drug [8]. Additionally, mucinated-honey was reported to have an excellence wound healing properties [8]. Mucin being a mucoaldhesive biological polymer, it has the potential to interact molecularly with gastrointestinal tract (GIT) system for effective binding to the wall of the GIT for prolong drug delivery at the site of absorption. Polyethylene glycol (PEG) is a non toxic polymer with good biocompatibility and has been widely use as drug

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carrier for oral and parenteral administration [9]. PEG steric hindrance has been widely speculated to protect protein drug from kidneys clearance and enzymatic degradation [10]. The interest in hybridization of mucin obtained from snail and chitosan grafted with PEG for oral insulin delivery arises from the existing reports on the individual polymer [8].

Recently, the race for an oral delivery of insulin using chitosan has received much needed attention among the pharmaceutical scientists. For instance, chitosan alone was investigated for oral insulin delivery and the author reported little success [11, 12]. Another work by Bin *et al.* [13], has reported the utilization of poly(lactic-co-glycolic acid) and chitosan for oral delivery of insulin. Similarly, Abdallah *et al.* [14], highlighted the effectiveness of chitosan when coated with hydroxyl propylmethyl cellulose phythalate (HPMCP) for oral insulin delivery. All these efforts are not without limitations such as early clearance of insulin, acidic degradation of insulin and dissolution of polymer carrier in the gastrointestinal tract by protonation. Additionally, positive result on the utilization of mucin for oral and rectal delivery [8] of insulin has been reported. Clinically, oral administration of drugs has been considered as the most convenient and comfortable route that eliminates pain caused by repeated injection of drugs [14, 15]. However, administration of insulin orally has been more of mirage than reality due to it instability and enzymatic degradation of the drug in the gastrointestinal tract system after oral administration. Additionally, the high molecular weight of insulin also inhibits its absorbtion through the tight junction of the intestinal system [15].

Strategies to overcome the challenges for oral insulin have been on the pipeline among the scientists focusing on drug delivery. For instance, cell-penetrating peptides (CPPs) have attracted increasing attentions as a promising vehicle for the systemic delivery of insulin across intestinal mucosa [6]. But the inability of CPPs to load high concentration of insulin and the poor stability of the formulation has been reported [6]. Previous studies were able to block the pores of polymeric PLGA MPs for the delivery of insulin, the inability of the microparticle to sustain the release of the drug has been considered as a big minus to the carrier system [16]. Similarly, Poly(lactic-*co*-glycolic acid) (PLGA) NPs have been extensively employed as a nanocarrier because of their ability to encapsulate and release drugs in a controlled manner, however limitations such as selectivity in their interaction with mucosal surfaces has been a major setback [17] Blanco MD).

The modification of natural polymer such as chitosan to prevent it protonation in an acidic medium of the gastrointestinal tract become very imperative considering the shortcoming of other strategies that has been previous evaluated. The objective of this research was to investigate the capability of chitosan–surface modified mucin microparticles to deliver the encapsulated insulin to the small intestine for improved absorption. Herein, we proposed a significant advance utilizing the mucin –chitosan for improve drug delivery. For instance, mucins are known to be the substrate on which mucoadhesive polymers attach [8], while chitosan is a nontoxic, biocompatible, cationic polysaccharide polymer that has gained much attention in the field of drug delivery and has special features of adhering to the mucosal surface and transiently opening the tight junctions (TJs) between epithelial cells by degradation or inactivation for drug absorbtion [18]. Chitosan has been used in micro- and nanoparticles formulations for controlled delivery of some bioactive materials due to its self gelation. It is our hope that mucin-chitosan composite would show a better capability to deliver sensitive molecule when used together thereby avoiding the short falls of individual polymer. Also, previous work shows two opposite surface charged had a better capability for drug carrier than similar charged [14, 15, 18].

Insulin-loaded microparticles were prepared via self gelation with chitosan as surface modifier for oral delivery. The prepared MPs were characterized *in vitro* with respect to particle size, shape, insulin loading, surface charge and release behaviour. The capability of the MPs to protect insulin against the harsh GIT environment was evaluated as a function of the hypoglycemic effects after oral administration in diabetic rats.

## 2. Materials and methods

### 2.1. Materials

The following materials were used: Polyethylene glycol 2000 (PEG-2000) ((Ph. Eur. Carl Roth GmbH Co. KG Karlsruhe, Germany), oleic acid and chitosan with molecular weight 10 kDa (Wako chemical Co, Japan) and alloxan (Sigma Chem, Ltd, USA) and insulin (Elly-Lily Company, USA), polycarbonate dialysis tube (Spectrum Labs., USA), glucometer meter (Accu-check, Roche USA). All others reagents used in this research were obtained from either Wako chemical. Co., Japan and Sigma–Aldrich, USA without further purification. Snail mucin was obtained from our laboratory, University of Nigeria, Nsukka Nigeria (UNN). Detail of snail extraction was previously reported [8]. Double distilled water (Lion water, UNN, Nigeria) was used throughout this research work.

### 2.2. Preparation of the microparticles

The preparation of microparticles was carried out by the multiple emulsion technique previously reported [19]. In brief, 5 ml of an aqueous solution of insulin (100 I.U/mL) was first emulsified under gentle magnetic stirring at 2000 rpm for 10 min, into 20 mL of 2 % chitosan solution in acetic acid (2 %). Approximately, 0.5 g of oleic acid in 10 ml of ethanol was gradually added to the pre-emulsion and homogenized at 10,000 rpm using an Ultra-Turrax T25 homogenizer (T25, IKA Germany). A mixture of 10 ml of 2 % (w/v) solution of polyvinyl alcohol (PVA), 5 ml of PEG (2 %) and 10 ml of 5 % (w/v) of insoluble snail mucin was added gradually and subjected to probe sonication at 60 W for 20 s (AT-500, probe sonicator, India). After evaporation of the ethanol from the preparation, the microparticles were separated by centrifugation at 10,000 g at 15 °C for 1h, freeze-dried and stored at 5 ± 3 °C. By adding increase concentration of 2, 4 and 6 % of chitosan, using the same procedure insulin-loaded microparticles (X-1, X-2, and X-3) were prepared. The drug free sample or unloaded (X-0) was similarly prepared.

### 2.3. Characterization of microparticles

#### 2.3.1. Recovery value

The recovery of the insulin-loaded microparticles was calculated using the Eq. (1).

$$\text{Percentage recovery} = \frac{A}{B + C} \times 100 \quad (1)$$

Where A represents the weight of the MPs prepared (g), B is the weight of insulin added (g) and C is the weight of the polymers and other excipients (g).

#### 2.3.2. Morphology investigations, particle size and surface charge

The morphological and the particle size of the MPs was evaluated using a photomicroscope (Hund®, Weltzlar, Germany) attached with a Motic camera 2.0 as earlier described by Momoh *et al.*, (2013). All evaluations on MPs were measured in triplicates. And the Zeta potential (ZP) was evaluated using a dynamic light scattering (DLS) using zetasizer nano (Malvern Zetasizer, UK).

#### 2.3.3. Thermal properties and FT-IR spectroscopic analyses

Thermotropic properties of the polymers and the insulin loaded microparticles were determined using a differential scanning calorimeter (DSC Q100 TA Instrument, Germany). Approximately 3–5 mg of each sample was weighed into an aluminium pan, hermetically sealed and the thermal properties investigated in the range of 20–220 °C at a heating rate of 5 °C/min. The cooling temperature was at the rate of 5–10 °C/min. An empty pan was used to establish the baselines, and all the thermograms were baseline-corrected.

Fourier Transform Infrared (FT-IR) spectroscopic analysis was

conducted on the drug and drug-loaded polymer using a Shimadzu FT-IR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000 to 400 $cm^{-1}$ with threshold of 1.303, sensitivity of 50 and resolution of 2 $cm^{-1}$ range.

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

In each case, a 20 mg of insulin-loaded MPs was added into a microconcentrator tube (Vivaspin® 6, Vivascience, Hanover, Germany) consisting of filter membrane with molecular weight cut off (MWCO) of 10,000 Da), a 10 ml deionized water was added into the tube containing the insulin-loaded MPs, thereafter it was centrifuged at 2000 rpm for 1 h and the supernatant collected, diluted and analyzed for insulin content spectrophotometrically (Unico 2102 PC UV/Vis Spectrophotometer, New York, USA) at a predetermined wavelength of 271 nm. The amount of insulin encapsulated in the MPs was calculated with reference to standard Beer-Lambert's plot for insulin sample to obtain the EE % using Eq. (2).

$$\text{EE}\ \ (\%) = \frac{\textit{Actual drug content}}{\textit{Theoritical drug content}} \times 100 \tag{2}$$

DLC expresses the ratio between the amount of insulin entrapped and total weight of the polymers as carrier. This was calculated using Eq. (3).

$$\text{DLC}\ \ (\%) = \frac{\textit{Amunt of insulin entrapped}}{\textit{Total weight of the polymers}} \times 100 \tag{3}$$

### 2.3.5. In vitro insulin release study

The polycarbonate dialysis tube was used for this investigation. Prior to the study, a 3 cm polycarbonate dialysis tube of molecular cut-off weight (MWCO) 10,000 g/mol (Spectra Spectrum Labs., USA), was soaked in the phosphate buffer solution (PBS) medium of pH 1.2 and 7.4 separately for 24 h. In each case, 20 mg of insulin-loaded microparticles was added into the tube, 3.0 ml of PBS was added and the tube was secured at both ends with thermo-resistant thread to prevent leakage. Thereafter, it was immersed in 250 ml of the dissolution medium maintained at 37 °C under agitation of 100 rpm provided by the magnetic stirrer. Approximately, 5 ml samples were withdrawn at interval of times and replaced with same volume (5 ml) of PBS maintained at the same temperature to maintain sink conditions throughout the release period. The withdrawn samples was filtered and analysed spectrophotometrically with reference to standard Beer-Lambert's plot of insulin at predetermined wavelength of 271 nm against the blank. All measurements were made in triplicates and values were plotted against time.

### 2.3.6. In vivo bioactivity studies

Wister rats of an average weight 190.0 ± 0.15 g were obtained from the animal house, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were kept in standard laboratory conditioned for a periods of two weeks before the commencement of the study. Diabetes was induced by peritoneal injection of alloxan dissolved in normal saline at a dose of 150 mg/kg as previously reported [18, 19]. After 5–7 days of the post administration, rats with blood glucose levels higher than 120 mg/dl were considered diabetic and were enlisted in the study. All animal experiment in this investigation was approved by the Departmental Committee on the uses of animal in line with the international approved guideline (ECC, 1986) [20].

### 2.3.7. Determination of insulin bioactivity

Thirty six diabetic rats were used in this investigation and were divided into four groups of nine rats (n = 9) per group following an earlier method [21]. Prior to the investigation, rats were fasted for 12 h before the administration of test samples and remained fasted during the experiment, but were allowed to have access to water. In this investigation, MPs with maximum drug encapsulation efficiency and release (X3) was chosen to evaluate the *in vivo* antidiabetics activity of the developed insulin-loaded MPs. Rats in group I received insulin-loaded MPs (batch X-3) containing equivalent to insulin dose of 40.0 I.U/kg body weight for each animal. Rats in group II were received oral free insulin solution (ins-sol) (40.0 I.U/kg), group III were administered oral saline water (5.0 ml) (negative control), and group IV were administered free insulin solution subcutaneous (SC) (4.0 I.U/kg), as a positive control, respectively according to their body weight. Treated rats were allowed access to water but no food was provided throughout the period of the investigation. Prior to administration of the test samples, blood basal glucose levels were established. After the administration of test agents, blood samples were collected from the tail vein at predetermined intervals of time and were analyzed for glucose levels using a glucometer meter (Accu-check, Roche USA). All experiment was carried out in triplicates and average reading was recorded.

### 2.3.8. Pharmacokinetics study

Pharmacokinetics study on plasma concentration of insulin- versus time curve after the oral administration of the formulation was investigated by the computerized curve-stripping program (PKAnalyst, software, Salt Lake City), and as previously reported elsewhere [9, 19]. Batch X3 showing highest release and encapsulation efficiency was selected in this investigation. Herein, three groups of diabetic rats (n = 9) were used in the investigation; Batch X3 of the insulin-loaded MPs (equivalent to 40 IU/kg) was administered orally to the group I, oral free insulin solution (40 IU/kg = 1.0 ml) to group II, and subcutaneous (sc) injection of free insulin solution (4.0 IU/kg) to group III. In the investigation, we used different concentration of insulin i. e the oral has the highest than subcutaneous injection, as it is established the oral faces a lot of GIT challenges such as fast pass effect among others. Blood samples were collected from the retro-orbital venous plexus using capillary tube, centrifuged at 4000 rpm for 30 min and the plasma was collected and the insulin concentration was quantified using a HPLC.

In brief, the HPLC system consisted of pump (PU- 2089, Jasco, Japan), UV–Vis detector (UV-2075, Jasco, Japan), auto sampler (AS- 206, Japan) and 250 mm × 4.6 mm column C18 particle diameter 5 μm (Shimadzu, Japan). Acetonitrite and phosphate buffer mixed in the ratio of 70: 30 at pH 7.4, was employed as the mobile phase and the flow rate was adjusted to 1 ml/min. The mobile phase filtered through 0.2 μm cellulose acetate membrane filter (Advantec, Japan) and degassed. The volume of injection was 20 μl and the total run time was 8 min. The detection wavelength was 227 nm and the column temperature was maintained at 25 °C.

The pharmacokinetics parameter was performed by the curve-stripping software. The area under the curve (AUC) was calculated by the linear trapezoidal rule. The maximum plasma concentration and the time of peak plasma concentration ($T_{max}$) were determined directly from the concentration-time data.

### 2.3.9. Data statistical analysis

The quantitative data in these investigations were performed in replicates (3–5 times) and results were expressed statistically as mean ± SD. One-way ANOVA and student's t test were performed on the data sets generated using SPSS [22]. Differences were considered significant at a level of $p < 0.05$.

## 3. Results and discussion

### 3.1. Recovery values

The recovery value of the MPs was very high in all the batches (>80 %) of the formulations. This indicates that the choice and methodologies used in the preparation were very reliable and the losses observed were insignificant ($p > 0.005$) enough to invalidate the procedure used in the preparation.

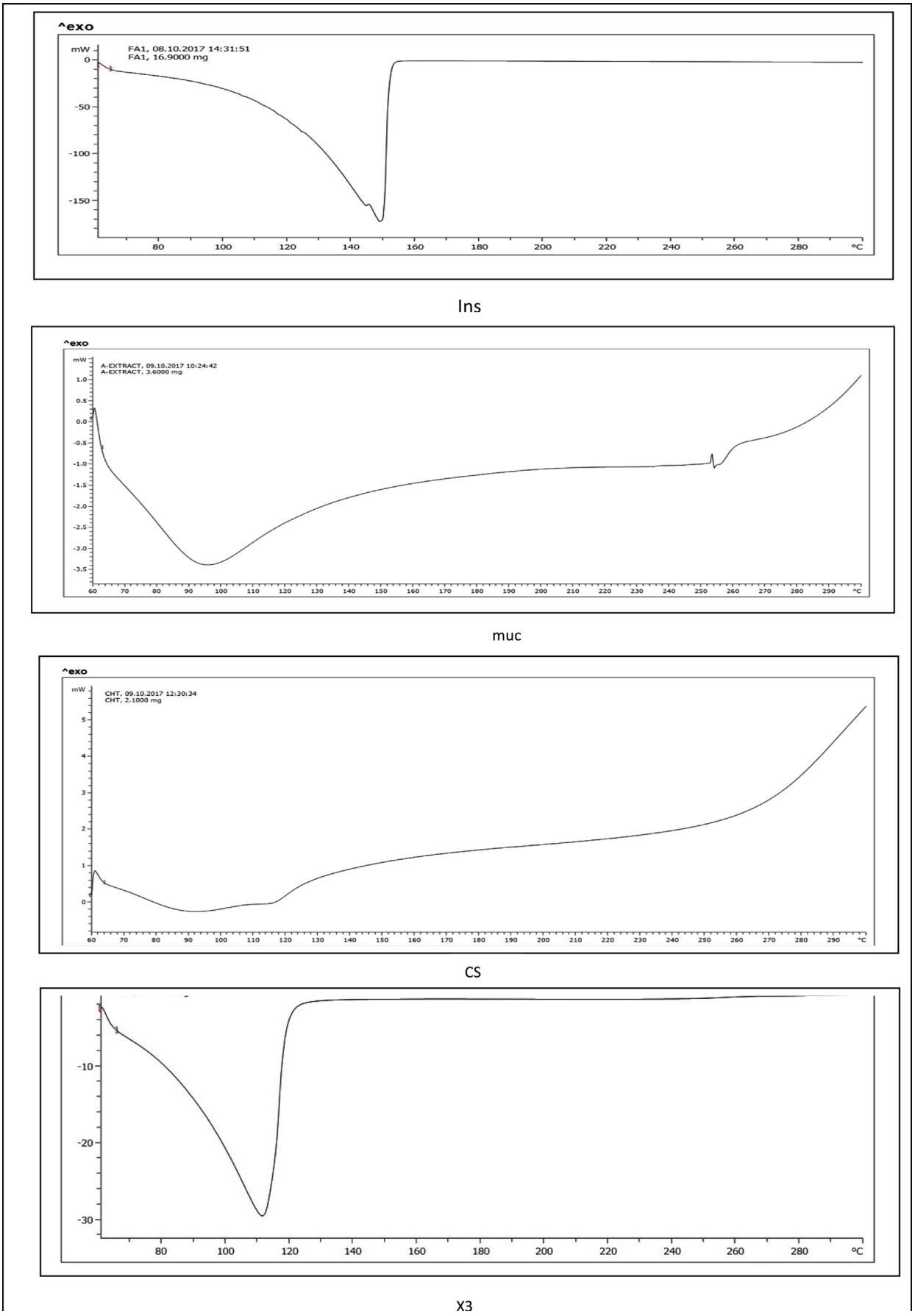


**Fig. 1.** DSC thermograms of chitosan (CS), mucin (MU), Insulin (Ins) and (X3) = insulin-loaded MP contain mucin with 6% chitosan solution.

### 3.2. Morphology, particle size and surface charge

Among the physicochemical properties, particle size and surface charge are of utmost relevance since they have direct relationship with the stability and acceptability of the drug delivery system for oral delivery. The particles are irregular in appearance, and free flowing as indicated by the result presented in Table 1. The particle size ranged from 37.5 ± 0.01 to 45.0 ± 0.12 μm. The insulin-loaded MPs showed higher particle size than the unloaded sample and the fabrication of the particle could be through ionotropic gelation. It was observed that majority of the particles were within the lower micrometer range and was dependent on the concentration of chitosan. As expected, the complexation of the polymer largely depended on their surface charges, which tends to influence greatly the net particle formation through ionotropic gelation. Previously studies reported that particle size is an important parameter to determine the pharmacodynamics and pharmacokinetics of any microparticle formulation. In addition, particle size influences the in vitro release, loading capacity and stability of the formulation [23]. In oral drug delivery, particle with small diameter enhances more absorption of the drug compared to very large particles that are not easily available for absorption [24]. In this investigation, we observed that the particle sizes (37.5–53.0 μm) were within the acceptable microsize range for formulation intended for oral administration. This result was in agreement with a similar report on insulin microparticles for oral delivery Philip *et al.*, [25]. From the surface charge shown in Table 1, it was observed that zeta potential values of the insulin-loaded microparticles were positive, indicating that chitosan was able to impart the positive moites on the surface of the microparticles and could encapsulate the insulin in the core of the polymer. In addition, positive charged surface of the microparticles will also enhances it adhesion to the mucosal wall of the intestine due to electrostatic attraction between the sialic content of mucin which carries negative charge, thus increase the mucoaldhesive activities and prolong the residence time of the formulation for better drug release and absorption. Based on zeta potential values of the formulations, the main interest on this work was on the insulin-loaded formulation which have zeta potential (ZP) values ± 30 mV and above. This range of value is an indicative of stability of the formulation. In this study, insulin-loaded batches shows a higher values as compare to the unloaded, this could be as a result of the incorporated drug (insulin) which interacted with the polymers or carriers thereby increase the repulsive forces to exceed the attractive forces, resulting in the difference in the zeta potential observed between the high zeta potential in loaded and low ZP in unloaded sample. It can also be explained that the absence of insulin in the unloaded sample gave room for the polymers to flocculate as a result of decrease in the inter and intra particulate distance and increase in the attractive forces above the repulsive forces, all these led to low or a decrease in zeta potential.

**Table 1**
Physicochemical parameters of insulin-loaded microparticles (n = 5).

| Batch | %EE | DLC | ZP (mV) | PS (μm) | RR (%) |
|---|---|---|---|---|---|
| X-1 | 80.1 ± 0.1 | 28.0 ± 0.0 | 29.0 ± 0.2 | 37.5 ± 0.3 | 88.1 ± 0.3 |
| X-2 | 82.1 ± 0.0 | 33.5 ± 0.0 | 32.0 ± 0.1 | 41.7 ± 0.1 | 96.1 ± 0.1 |
| X-3 | 89.5 ± 0.1 | 33.1 ± 0.1 | 37.0 ± 0.6 | 43.0 ± 0.1 | 97.2 ± 0.7 |
| X-0 | – | – | 21.8 ± 0.1 | 41.8 ± 0.0 | 89.2 ± 0.1 |

Key: EE = encapsulation efficiency, DLC = drug loading capacity, ZP = zeta potential, PS = particle size, and RR = recovery, of MPs formulations, (n = 5). Note: (X1)insulin-loaded MP contain 2% chitosan, (X2)insulin-loaded MP contain 4% chitosan and (X3)insulin-loaded MP contain 6% chitosan.

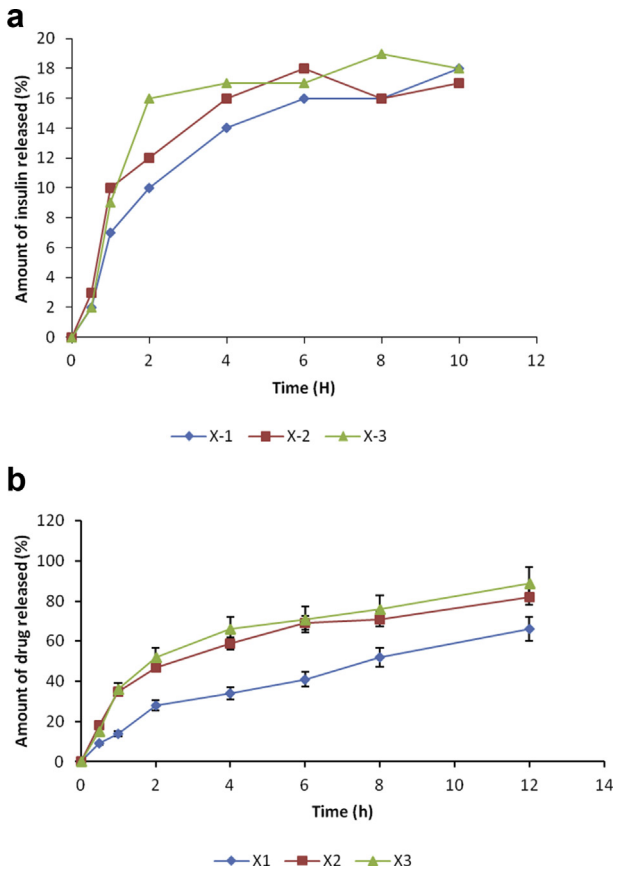


**Fig. 2.** (a and b) *In vitro* drug release of insulin-loaded microparticles in acidic pH 1.2 (a) and phosphate buffer pH 7.2 (b) (n = 5). Note: (X1) = insulin-loaded MP contain 2% chitosan, (X2) = insulin-loaded MP contain 4% chitosan and(X3) = insulin-loaded MP contain 6% chitosan.

### 3.3. Encapsulation and drug loading capacity

There was high drug encapsulation (>80 %) in all the batches of the formulation as shown in Table 1. It was observed that the ratio of the polymer did not significantly affect the encapsulation efficiency of the drug. However, it was reported that high EE % is achieved when the polymer and the drug has opposing charge [26]. Encouragingly, the ionic interaction of the opposite charges between insulin and the polymers during microparticles formation would play an important role in drug encapsulation and drug loading into the microparticles. Additionally, despite the solubility of the drug in aqueous medium, the steric hindrance network of the PEG-2000 would also give protection for the migration of the drug out of the particle. This could be one of the reasons why we observed no burst effect in this formulation during the *in vitro* release.

### 3.4. Thermal properties

The changes in the thermal properties of the polymers and the formulation as shown by the DSC are depicted in Fig. 1. The DSC results of mucin showed two melting endothermic peak at 94.88 °C and 254.94 °C with corresponding enthalpy of -3.39 and -1.02 mw/mg, respectively. This indicates its crystalline nature while the DSC thermogram of chitosan showed no obvious melting peak as illustrated in Fig. 1 (CS). The DSC thermogram of pure insulin solution showed a melting peak at 136.17 °C with corresponding enthalpy of -32.56 mw/mg. The DSC thermogram of insulin-loaded MPs showed different melting peaks and thermal properties as depicted in representative of the insulin MPs Fig. 1 (X3) For instance batch X1 and X2 (Figure not shown) showed melting peak of 146.39 °C and 117.77 °C with corresponding enthalpy of -108.50 and 19.04 mw/mg, respectively. Compared the thermal properties of pure insulin, chitosan, mucin with those of insulin-loaded MPs, it indicates insulin-MPs gave lower melting point than individual polymers. Previous work has shown that decrease in melting point values indicates less ordered crystal structures, it follows that all insulin-loaded MPs are less crystalline indicates that they existed in amorphous form in the microparticle encapsulating the drug. Additionally, there are no significant ($p > 0.005$) changes in the heat capacity of the different formulation and no formation of double peak in all the preparation. This clearly shows that the polymer formed a new entity with properties different from individual polymer with no obvious polymer-drug interaction observed.

### 3.5. FT-IR spectra analysis

The FT-IR spectra of X-2 and X-3 of the insulin-loaded MPs and unloaded X-0 MPs were carried out. FTIR spectrum of pure insulin sample showed a principal characteristic peaks at 3539.38 $cm^{-1}$ and 3446.74 $cm^{-1}$ (O–H stretch of alcohol), 3446.74 $cm^{-1}$ (N–H stretch of amine), 2956.52 $cm^{-1}$ and 2863.88 $cm^{-1}$ (C–H stretch of alky), 2640.00 $cm^{-1}$

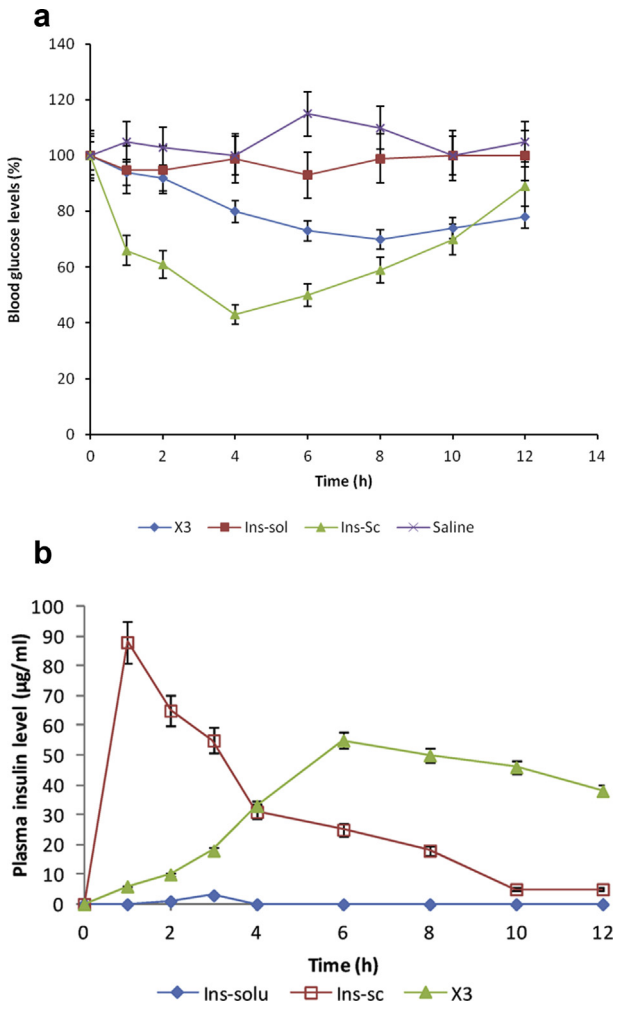


**Fig. 3.** a, b (3a) Percentage blood glucose levels after oral administration of test agent (X3) insulin-loaded MPs, insulin solution (ins-sol), subcutaneous (ins-sc) and saline. (3b). Plasma concentration of insulin after orally administered (X3) = Ins-MPs contain 6% chitosan, insulin solution (sc) and insulin solution (orally), mean ± S.D., n = 5).

(O–H stretch of carboxylic acid), 2088.02 $cm^{-1}$ (C=C stretch of alky), 1586.22 $cm^{-1}$ (C=O stretch of carboxylic acid) and 1454.98 $cm^{-1}$ (C=C stretch of benzene ring). For FTIR spectrum of insulin-loaded MPs, showed principal characteristic absorption peak at 3589.56 $cm^{-1}$ and 3292.35 $cm^{-1}$ (O–H stretch of alcohol), 3381.12 $cm^{-1}$ and 3292.35 $cm^{-1}$ (N–H stretch of amine), 2925.64 $cm^{-1}$ (C–H stretch of alky), strong wavelength were observed at two different wave 2612.98 $cm^{-1}$ and 2520.34$cm^{-1}$ (O–H stretch of carboxylic acid), 2061.00 $cm^{-1}$ (C=C stretch of alky) and 1563.06 $cm^{-1}$ (C=O stretch of carboxylic acid). FTIR analysis of chitosan, insulin and insulin-loaded MPs showed FTIR spectra similar to that of the individual material and suggests the absence of new chemical identity molecules formed and thus, suggests chemical compatibility and also the absence of chemical alterations between the drug and excipients used in formulating the microparticles. This is in agreement with the transition peaks and the enthalpy seen in the DSC thermograph as shown in the DSC section of the study (Fig. 1). The transformation of the crystalline state to amorphous state could be explained based on the stretching and the absence of any losses of prominent bands in the loaded sample. These clearly indicate that there was no incompatibility between the polymer and the drug. However, the stretching could be as a result of the electrostatic interaction between the surface charges of the polymer and the drug, which ultimately enhance the mucoaldhesive and prolong the residence time of the drug at the absorption site.

### 3.6. *In vitro release study*

As shown in (Fig. 2a and b), the *in vitro* release profiles of insulin in phosphate buffer of pH 1.2 and 7.2, respectively over a period of 10–12 h. Interestingly, the insulin release from microparticles was pH-dependent which was retarded at pH 1.2 (<20 %) as there was in- significant insulin release at acidic pH 1.2 Fig. 2a. However, there was a significant release of insulin in phosphate buffer at pH 7.2 (Fig. 2b), indicating the protection of significant amount of insulin encapsulated in the microparticle from the acidic environment of the stomach by the polymers/carriers and possible release of drug in the intestine, where uptake and absorption of insulin take place by the intestinal cells. Formulation X1, X2 and X-3 gave the maximum release of 79.4, 76.6 and 68.2 % at 12 h, respectively. There was significant difference ($p > 0.05$) in the release profiles of the various batches of the preparation. Studies have shown that the release of drug depends largely on the concentration of drug loaded and the polymer used as the carrier [26, 27]. In this study, the drug loaded into the microparticles were the same in all the batches of the formulation, however, there was a variation in the concentration of the surface modifier (chitosan), hence the variation in the release could be ascribed to the chitosan. Drug release from microparticles should theoretically be slower as the concentration of chitosan is increased because of an increase in the path length through which the drug has to diffuse [28]. However, results obtained indicate that increase in the concentration of chitosan caused an increased in the path length and thereafter a decrease in the early drug release from the microparticles formulations, but sustain the release after complete rupture of the particles thereby showing the highest release. In other words, batch X-3 showed a significant and sustained release profile for 12 h as compared to other batches of the formulation due to the strong electrostatic interaction between insulin molecules and the increased in the chitosan polymer [27]. This result was consistence with the report of similar work where the increase in the cationic group on thiolated trimethyl chitosan showed a sustained release of insulin [29]. On the contrary, previous report indicates that insulin-chitosan loaded MPs rapidly dissociated in acidic medium due to protonation of the chitosan[3]. A similar result was also reported on the stability of chitosan/TPP [17]. In this study, insulin-loaded MPs was fortified with mucin and showed an improve in the stability at a lower pH with low insulin release (<20 %) as against the nearly 100 % release within 10 min when unprotected chitosan was used as reported [30]. The results from the in vitro release study are a strong indication that mucin modified chitosan showed a promising carrier for oral delivery of anionic/peptide drug like insulin.

### 3.7. *Determination of glucose bioactivities*

The bioactivities of the insulin-loaded MPs are investigated in alloxan induced diabetes rats as model animal. As shown in Fig. 3a, there was no observable reduction on blood glucose levels when saline was orally administered to the rats. It could be observed that rats in this group maintained a high blood glucose levels throughout the evaluation period and some of the rats died. Similarly, the insulin solution administered orally also shows no remarkable decrease in the blood glucose levels in all the rats. This clearly shows that oral insulin solution could not exert the expected decrease in blood glucose levels, an indication that absorption of insulin from the GIT was not possible due to absence of carrier or protective agents. Thus, the transient decreased of 1–2 % after the administration (Fig. 3) was not significant enough to assume that there was good absorption of insulin from the GIT after oral administration. As expected, subcutaneous injection of insulin (positive control), show a sharp and significant decrease in the blood glucose level and was sustain for over 4 h. The effects was maximum at 4 h with >60 % decrease in blood glucose levels being observed. However, after 5 h of the effect, there was a steady increase in the blood glucose levels toward initial level of 100 %. The observed increase in the glucose level after 5 h indicate that there was rapid clearance of the insulin from the circulation which could be attributed to factors such as short half-life and poor sustain release as earlier reported by Lei *et al.*, [11, 13]. Encouragingly, there was a significant reduction in the blood glucose levels after oral administration of batch X-3, though the rate of decrease in blood glucose levels and the onset of action was not comparable to subcutaneous injection of insulin, but significantly better than free oral insulin solution. However, despite the slow onset of action of the test sample, it could be observed that the formulation showed a persistent decrease in blood glucose levels for a period of 8 h and further maintains steady decrease for 12 h, as compare to the subcutaneous administration that lasted only for 4 h as depicted in Fig. 3. Previous work on free chitosan, showed a similar result with our findings [30]. It was observed that the decreased in blood glucose levels by our formulation is higher and sustained for a longer time as compared to the earlier report [31], where the authors opined that protonation of chitosan in acidic environment was responsible for the poor decreased in blood glucose levels [15].

In this our investigation, it can be argued that mucin was able to prevent the protonation of chitosan due to it entanglement of network with chitosan thereby prevent early release of the encapsulated insulin. Additionally, combination of these polymers could synergistically open the tight junctions (TJs) by degradation or inactivation thereby resulting in paracellular membrane transport which favours more insulin absorption into the system.

### 3.8. *Pharmacokinetics study*

The plasma concentrations of insulin over the 12 h study period are depicted in Fig. 3b, and pharmacokinetic parameters are given in Table 2. Pharmacokinetics study of the presence of insulin in the plasma was investigated over 12 h after fasted rats were orally administered the insulin microparticles (X3), insulin solution (oral) and insulin solution (SC) at a dose of 5 IU/kg, 50 IU/kg and 50 IU/kg, respectively. The concentration of insulin in blood peaked after oral administration of insulin-loaded microparticles (X3) at 5 h and slowly decreased over a period of 12 h. In contrast, insulin solution administered SC shows a rapid peak at maximum within 1h after administration and was followed by fast decrease in the plasma concentration of insulin SC as compared to the formulation (X3) administered orally. On the insulin solution without carrier show no significant ($p > 0.05$) peak after oral administration. As expected the $T_{max}$ of the subcutaneous administered insulin was faster than the orally administered formulation. However, the AUC of the

**Table 2**
Pharmacokinetic parameters of plasma concentration of insulin after oral administration of insulin-loaded microparticles (n = 5).

| Pharmacokinetic parameters | Ins-solu (oral) | Ins-solu (sc) | Ins-MPs (X3) |
|---|---|---|---|
| $C_{max}$ (μg/ml) | 4.2 ± 6548 | 88.0 ± 22.3 | 55.0 ± 17.1 |
| $T_{max}$ (h) | 3.0 | 1.0 | 6.0 |
| AUC (μg $mL^{-1}$ $h^{-1}$) | 12.11 ± 16.2 | 342.56 ± 30.9 | 379.23 ± 13.1 |

Note: Ins-MPs (X3) = insulin-loaded microparticle contain 6 % chitosan, insulin solution (orally) = insulin solution administered orally to the rats, and insulin solution (sc) = insulin solution administered subcutaneously to the rats; $C_{max}$ = Maximum blood concentration of insulin, $T_{max}$ = Time for maximum peak of insulin and AUC = The area under.

formulated insulin-loaded microparticles was significantly ($p < 0.05$) higher than those of insulin solution administered subcutaneously. This result could be attributed to the rapid decreased in the SC administration as a result of fast clearance of insulin from the systemic circulation. Interestingly, the oral administered insulin-loaded microparticles show a prolong effect on the blood system which could be due to the effect of the carriers as they are able to release the insulin in sustained manner.

### 3.9. Stability study after six months of storage

The resuts of the parameters evaluated after six months of storage shows no significant difference from the data obtained in the evalaution carried out within 48 h of the preparation. The encapsulation efficiency and drug loading ranged from 78.8 ± 0.4 and 31.2 ± 0.2 respectively, indicates that the optimized formulation (batch X-3) is very stable after six months. The result further confirmed that the selected polymers and the procedures used in the formulation are very reliable and could be further exploring for possible oral insulin deilvery.

## 4. Conclusions

In the present study, chitosan modified snail mucin microparticles were developed for oral delivery of insulin. The prepared insulin-loaded microparticle provided the particle size in low micrometers (<440 μm) suitable size for oral absorption. The drug encapsulation (>80 %) was siginifcantly high enough and it was released in a sustained over 10 h *in vitro*. The positive zeta potential, indicated the drug was encapsulated in the core of the polymer. The hypoglycemic activities of insulin was retained, with significant reduction (>50 %) in blood glucose levels and ensured prolonged effects of over 10 h *in vivo*. It is likely that our methodology in this investigation utilizing snail mucin has demonstrate the added value of microparticle and has the capability to be use in peptides drug development like insulin to overcome the barriers for oral insulin delivery.

## Declarations

### Author contribution statement

Mumuni Momoh, Ofokansi C. Kenneth, Anthony Attama, Franklin Kenechukwu, Omeje C. Ernest, Adedokun M. Oluseun: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Barikisu Abdulmumin, Youngson Darlington C.: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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