Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

# Novel Mucoadhesive Microspheres Prepared by Emulsification-Coacervation Technique for Oral Insulin Delivery: Effect of Polymer Concentration on Drug Pharmacodynamics

MOMOH MA, KENECHUKWU FC*, MGBARAMUKO U, OFOKANSI KC

Drug Delivery and Nanomedicines Research Group, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.

**ARTICLE DETAILS**

*Article history:*
Received on 14 February 2017
Modified on 29 June 2017
Accepted on 15 July 2017

*Keywords:*
Blood Glucose Reduction,
Insulin,
Oral Administration,
Microspheres,
Eudragit® RL 100.

**ABSTRACT**

The purpose of this study was to formulate novel mucoadhesive Eudragit microspheres by emulsification-coacervation technique for oral insulin delivery and evaluate the effect of polymer concentration on the anti-diabetic property of the entrapped drug. Mucoadhesive insulin-loaded microspheres containing magnesium stearate and varying proportions of Eudragit® RL 100 were prepared by emulsification-coacervation technique and evaluated for physicochemical performance and *in vivo* hypoglycemic effect in alloxan-induced diabetic rats after oral administration. Stable, spherical, brownish, discrete, free flowing and mucoadhesive insulin-loaded microspheres with size range (14.20±0.30–19.80±0.60 µm) and loading efficiency (74.55 ± 1.05 – 75.90 ± 1.94 %) were formed. Reduction in the blood glucose level by the orally administered insulin-loaded microspheres, which was significantly ($p \leq 0.05$) higher than subcutaneous insulin and oral insulin solution, indicates that the former could be effective alternative for oral delivery of insulin. This study has shown that oral delivery of insulin for effective control of blood glucose could be possible using Eudragit® RL100 entrapped microspheres prepared by emulsification technique.

© KESS All rights reserved

## INTRODUCTION

Diabetes mellitus, a hereditary metabolic disease characterized by hyperglycaemia and eventual glycosuria, is caused by the inability of tissues to carry out normal metabolism of carbohydrates, fats and proteins, due to an absolute or relative lack of insulin [1-3]. Its complications are responsible for excess morbidity and mortality, loss of independence, and reduced quality of life. The main goal of diabetes management is to restore carbohydrate metabolism to as close to a normal state as possible [4]. To achieve this goal, individuals with an absolute deficiency of insulin require insulin replacement therapy. Insulin resistance, in contrast, can be corrected by dietary modifications and exercises. In other words, insulin replacement therapy for many decades, has been used as a first line agent in type I diabetes and sometimes in the treatment of type II diabetes, where oral hypoglycaemic agents combined with diet and exercises fail to achieve appropriate metabolic control [5-8]. However, there are limitations or problems encountered during subcutaneous insulin injection, including local discomfort, pain, allergic reactions, hyperinsulinaemia, as well as inconvenience of multiple injections, and occasional hypoglycaemia as a result of overdose [6, 9-10]. Because of these problems, novel approaches for insulin delivery are being explored, including oral, rectal, pulmonary, uterine, and ocular delivery as well as subcutaneous implants. Delivery options that use dermal, nasal, and transdermal approaches have also been explored, with current and more emphasis on oral delivery system [5-8, 11-14]. The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides like insulin by mouth. In addition, administration of insulin via the oral route will help eliminate the pain caused by injection, psychological barriers linked with

***Author for Correspondence:***
*Email:* frankline.kenechukwu@unn.edu.ng
chiafrankduff@yahoo.com

multiple daily injections, such as needle anxiety and possible infections [15,16].

Microspheres are small spherical particles having their diameter in range of 1-1000 microns with different densities. They are made up of natural and synthetic substances like polymers, or other natural polysaccharides like starches and even waxes, gum, proteins and fats are used as drug carrier matrices for drug delivery [17-20]. Eudragits® are polymeric substances the physicochemical properties of which are determined mainly by their functional groups [21]. Eudragit® polymers are more favoured in the formulation of pH-sensitive drug molecules. The obvious advantages of these polymers include pH-dependent release profiles, encapsulation of high amount of drug, release of the incorporated drug in controlled manner and high level of stability [22]. Generally, Eudragits® are copolymers of acrylic and methacrylic acid esters with quarternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable [23]. Eudragit® RL 100, which is pH-independent and mainly releases its drug content in the intestine, was used for this research. Variation in the quantity of quaternary ammonium group causes variation in their permeability characteristics [24], which could be utilized to improve the impermeability characteristics of poorly permeable biomolecules such as insulin.

Consequently, the aim of this study was to utilize the enteric properties of Eudragit® RL100 in a microsphere drug delivery system for oral administration of insulin. Oral deliveries of insulin using various carriers have been investigated by various researchers [25-38]. The novelty of the work lies on the use of Eudragit ® RL 100 and magnesium stearate (a hydrophobic droplet stabilizer) in combination to improve the controlled release effect of insulin-loaded Eudragit entrapped microspheres prepared by the emulsification-coacervation technique.

## MATERIALS AND METHODS

### Materials

The materials used include methacrylic acid copolymer (Eudragit® RL100) (BASF Chemical Industry Germany), sorbitan monostearate (Span 60) (Merck, Germany), Liquid paraffin (Moko Pharm. Ltd., Nigeria), magnesium stearate, n-hexane, acetonitrile and perchloric acid (BDH, England), potassium dihydrogen phosphate (monobasic potassium phosphate), sodium hydroxide, concentrated hydrochloric acid and acetone (Sigma-Aldrich, USA), distilled water (Freshly prepared in Biochemistry lab, UNN), insulin (Humulin 70/30) (Lilly, Egypt). These materials were used as procured from the manufacturers without further purification. All other reagents were analytical grade and used as such. The animal experiments complied with the regulations of the Committee on Ethics on the Use of Laboratory Animals of the University of Nigeria in accordance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

### Methods

#### Formulation of Insulin-Loaded Microspheres

Insulin microspheres were prepared according to oil-in-oil emulsification-coacervation method using Eudragit® RL100 polymer which was dissolved in 12.5 ml of acetone in a 250 ml beaker with stirring at room temperature. Insulin (0.5 ml of 100 International Unit (IU)) and magnesium stearate (0.1 g) were dispersed in the polymer solution. The resulting milky white dispersion was added drop wise into a beaker containing a mixture of liquid paraffin (50 ml) and span 60 (0.5 g) and homogenized using a paddle stirrer (Remi Instruments, Mumbai, India) at 500 rpm for 2 h. The resulting microspheres were harvested by filtration, washed severally with n-hexane until they were completely free of oil. The microspheres were dried at room temperature and stored at 4 $^{o}$C until used. Three batches of the microspheres were prepared for different amounts of the polymer and a control was also prepared using the above method without insulin, as shown below in Table 1.

#### Determination of Percentage Yield

The formed microspheres were recovered and weighed accurately. The yield of microspheres was determined by comparing the whole weight of formed microspheres against the combined weight of the copolymer and drug using the equation below:

$$Yield\ (\%) = \frac{Total\ weight\ of\ the\ microsphere\ obtained}{Total\ weight\ of\ the\ excipients\ and\ the\ drug\ used} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

#### Thermal Analysis

Briefly, the thermal properties of insulin, Eudragit® RL 100 and drug-loaded microspheres were studied using a differential scanning calorimeter (DSC 204 F1 Netzch, Germany) to evaluate any possible drug-polymer interaction.

**Table 1:** Formulation compositions of the microspheres

| Formulation code | Insulin (ml of 100 IU) | Eudragit® RL 100 (g) | Magnesium stearate (g) | Drug: Polymer ratio |
|---|---|---|---|---|
| **$U_0$** | 0.0 | 2 | 0.1 | 0:1 |
| **$U_1$** | 0.5 | 2 | 0.1 | 1:4 |
| **$U_2$** | 0.5 | 3 | 0.1 | 1:6 |
| **$U_3$** | 0.5 | 4 | 0.1 | 1:8 |

$U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100 while $U_0$ is the unloaded microspheres

The analysis was performed at a heating rate of 10° C/min from 10° C to 400° C temperature range under an inert nitrogen atmosphere with a flow rate of 20 ml/min.

**Quantitative Determination Of Insulin**

The insulin content of the microspheres was determined using a high performance liquid chromatography (HPLC). The machine consisted of an Agilent 1100 series programmable separating module, quartenary pump G 1311 A (Agilent technoloy, USA), an auto degasser G1322A, and a variable wavelength detector G1314A. The column was a reverse phase ODS (C-18, 5µm 4.6 x 250 mm, Supercosol USA) equipped with a guard. The mobile phase consisted of acetonitrile and water (10:90), perchloric acid was used to adjust the pH to 3. The flow was set at 0.8 ml/min and the chromatograms recorded at 280 nm.

**Insulin Loading Efficiency**

A 10 mg quantity of microspheres was dispersed in 10 ml of simulated intestinal fluid (SIF, pH 7.2). The dispersion was allowed to stand for 2 h after which it was mixed with a vortex mixer (Remi Instruments, Mumbai, India) for 5 min and then centrifuged at 4000 rpm for 10 min. The amount of insulin contained in each batch of the formulations was determined by the HPLC method. The drug loading efficiency was then determined by evaluating with equation 2 [8,11].

$$ILE = \frac{AD}{TD} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, ILE is insulin loading efficiency, AD is actual amount of insulin in microspheres and TD is the theoretical amount of insulin in microspheres.

**Morphology and Particle Size Analysis**

The size and morphology of the microspheres were analyzed by computerized image analysis using samples mounted on a glass slide (Marinfield, Germany). These samples were dispersed in little quantity of liquid paraffin and smeared on the slide using a glass rod. It was then covered with a cover slip and viewed with a photomicroscope (Hund®, Weltzlar, Germany) attached with a digital camera at a magnification of 1000x. With the aid of the software in the photomicroscope, the particle morphologies were observed and photomicrographs taken. The sizes of the particles were measured (n = 30) and average taken.

**Mucoadhesiveness of the Microspheres**

The mucoadhesive properties of the microspheres were evaluated by the *in vitro* wash-off test as reported by Ofokansi and Adikwu [39]. The apparatus used for this study was designed to give reproducible results. A 200 mg quantity of the microspheres was weighed accurately and placed on an 8.5 cm long porcine ileum and allowed to interact with and adhere to the surface of the ileum. A 50 ml portion of simulated intestinal fluid (SIF) was poured into a separating funnel, clamped to a retort stand, and allowed to run over the microspheres on the porcine ileum. The microspheres that detached from the ileum were collected, dried and weighed. This was repeated for all batches. The percentage mucoadhesion for each batch was calculated using the formula below:

$$\text{Percentage mucoadhesion} = \frac{\text{weight applied} - \text{weight detach}}{\text{weight applied}} \text{X100} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

**Pharmacodynamic Studies**

***Induction of Diabetes***

Rats weighing between 180 – 280 g were purchased from the Department of Biochemistry, University of Nigeria, Nsukka. The rats were all kept in standard and conditioned animal cages and left for one week to acclimatize to the new

laboratory environment while being fed with standard laboratory diet. Diabetes was induced by intravenous injection of alloxan dissolved in normal saline through the marginal ear vein at a dose of 120 mg/kg [40]. After 3–5 days of the alloxan treatment, rats with frequent urination, loss of weight, and blood glucose levels higher than 120 mg/dL were considered diabetic and selected for the study. The rats were monitored for persistent blood glucose elevation for 5 days [17]. Before testing, animals were fasted overnight with free access to water.

### *In Vivo Anti-Diabetic Study*

Thirty five Wistar rats (either sex) were used for the evaluation of the anti-diabetic effects of the formulations. In each case, the animals were fasted for 12 h prior to oral drug administration. Rats were divided into seven groups of five animals each. The diabetic rats were administered oral insulin-loaded microspheres encapsulated in hard gelatin capsules. In order to evaluate the effect of administration on hyperglycaemia, distilled water, insulin solution and unloaded microspheres were orally administered as negative controls, while subcutaneous injection of insulin was given as positive control. Blood samples were withdrawn from the tail vein at predetermined intervals of 0, 0.5, 1, 2, 4, 6, 8, 10 and 12 h, and blood glucose levels were measured using Accu-check glucometer. (Switzerland). Food and water intake as well as urine output of the animals were measured and monitored in the course of the study.

### Statistical Analysis

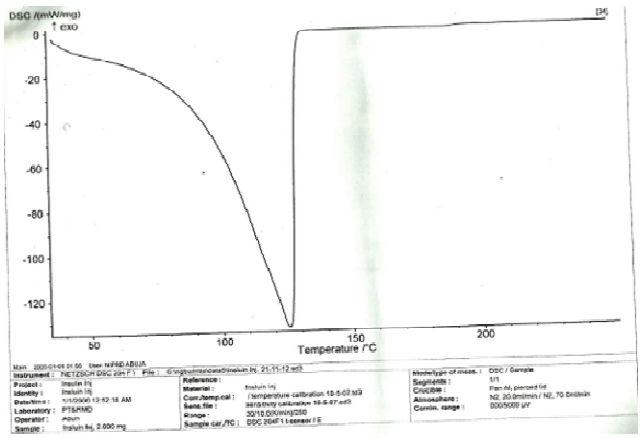
All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± SD. ANOVA and student's t-test were performed on the data sets generated using SPSS. Differences were considered significant for $p \leq 0.05$.

## RESULTS AND DISCUSSION

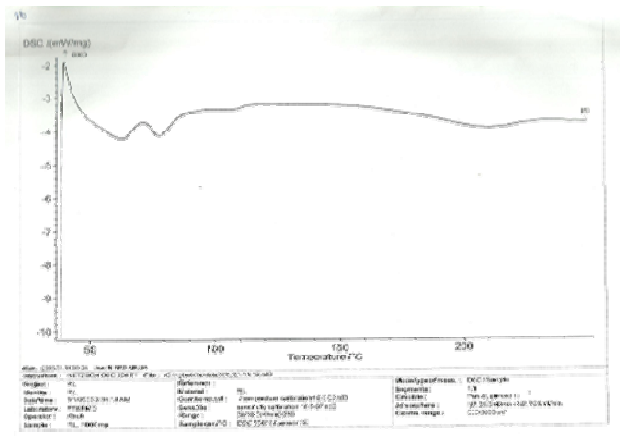
The percentage yield of all the batches ranged from 50.93 to 77.62 %, with batch $U_2$ having the highest percentage yield (Table 2). The yield of the microspheres were generally high. In this study, mucoadhesive insulin-loaded microspheres containing magnesium stearate and varying proportions of Eudragit® RL 100 were prepared by emulsification-coacervation technique and evaluated for oral insulin delivery. There was no evidence of correlation between the drug:polymer ratio used in the formulation of microspheres and the microspheres yield. In all cases, the yield of the microspheres from all the formulations were generally high indicating that the formulation procedures and parameters employed in formulating the microspheres are very effective and efficient. The percentage loss was low, and this might arise during the filtration, transferring or drying.

Fig. 1 shows the thermograms of insulin and the microspheres while Table 3 presents the thermal properties of insulin and the formulations. DSC result of insulin showed a melting peak of 125 $^{o}$C with an enthalpy of – 132 mW/mg (Fig. 1a). The unloaded microspheres (Fig. 1b) showed two melting peaks (62.7 and 78.2 $^{o}$C) with corresponding enthalpies of - 4.216 and - 4.131 mW/mg. The DSC thermograms of insulin-loaded microspheres showed different melting peaks and thermal properties, as depicted in Fig. 1c–e. The results showed that with the exemption of batch $U_1$, which has a melting peak of 63.6 $^{o}$C with a corresponding enthalpy of -4.131 mW/mg, all the drug-loaded microspheres showed two melting peaks [64.2 and 85.6 $^{o}$C (batch $U_2$), 63.9 and 81.6 $^{o}$C (batch $U_3$)] with corresponding enthalpies of -8.821 and -7.662 mW/mg (batch $U_2$), -3.360 and -2.561 mW/mg (batch $U_3$). The results of the differential scanning calorimetry (DSC) analysis showed that insulin is properly solubilized in the microspheres, since higher melting point values indicate more ordered crystal structures, consistent with previous studies [14, 40]. More so, the physicochemical compatibility of the drug and the polymer studied by DSC suggested absence of any incompatibility. The results revealed the compatibility of insulin and the polymer (Eudragit® RL 100). In addition, the formulations (drug-loaded microspheres) gave lower melting point values than insulin (Fig. 1f), implying that insulin existed in amorphous state in the formulations and also was properly solubilized in the microspheres [17].

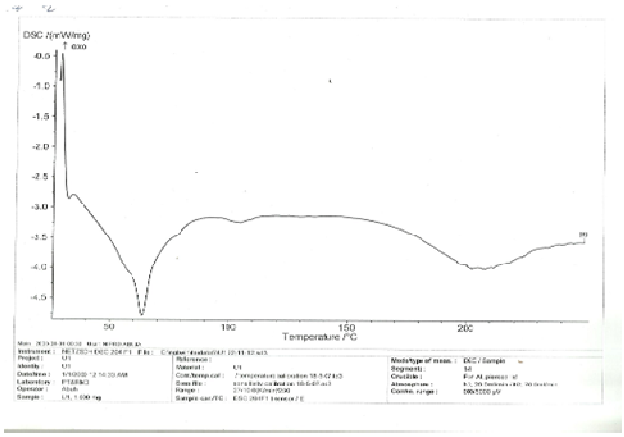
The drug loading efficiency is shown in Table 2. The results indicate that there was no general pattern of drug entrapment with regards to increasing proportions of Eudragit® RL100 used in preparing the microspheres. However, microspheres prepared with 3 g of Eudragit® RL100 entrapped greater amount of insulin in comparison with the rest of the microspheres batches. The drug loading efficiency is an important variable for assessing the drug loading capacity of microspheres and their drug release profiles.



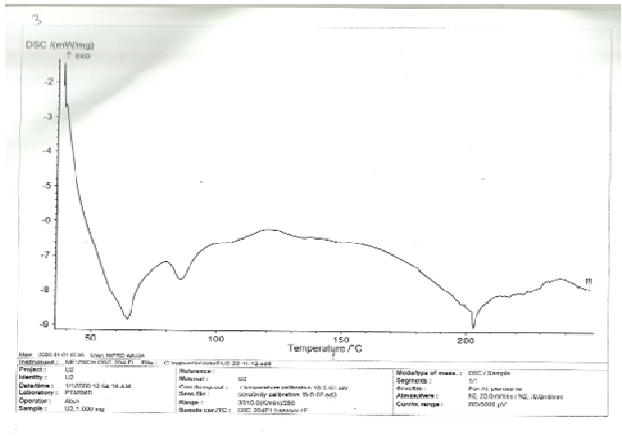

**(1a)**



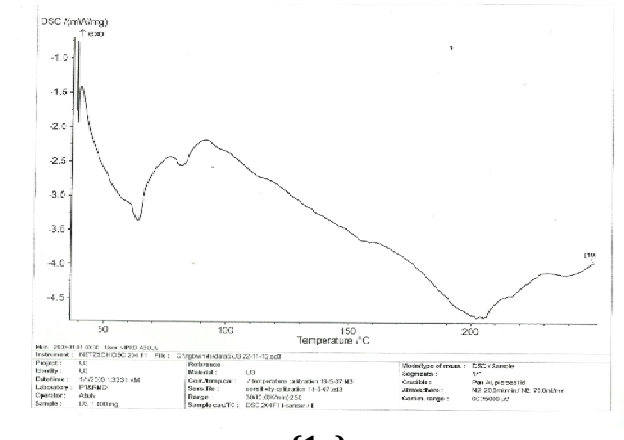

**(1b)**



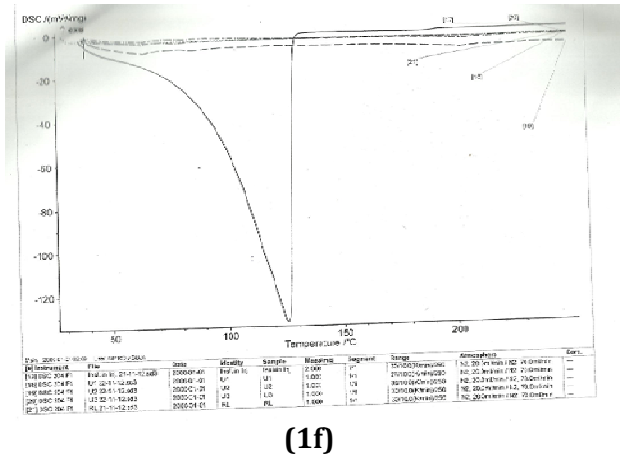

**(1c)**




**(1d)**




**(1e)**




**(1f)**

**Figure 1:** DSC thermogram of (a) insulin (b) unloaded Eudragit ® RL 100 microspheres (batch $U_0$) (c) insulin-loaded microspheres batch $U_1$(d) batch $U_2$ (e) batch $U_3$(f) formulations overlayed.
**Keys:** $U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100 while $U_0$ is the unloaded microspheres

**Table 2:** Some physicochemical properties of the microspheres

| Batch code | Yield (%) | Size (µm) [a,b] | EE (%)[a,b] | Muco- adhesion (%)[a,b] |
|---|---|---|---|---|
| **$U_0$** | 50.93 | 13.5±0.9 | - | 75±2.3 |
| **$U_1$** | 63.99 | 14.2±0.3 | 75.23±2.10 | 70±2.5 |
| **$U_2$** | 77.62 | 17.6±0.5 | 75.90±1.94 | 80±3.0 |
| **$U_3$** | 71.16 | 19.8±0.6 | 74.55±1.05 | 60±2.9 |

[a]Mean ± SD, [b]n = 3; $U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100 while $U_0$ is the unloaded microspheres.

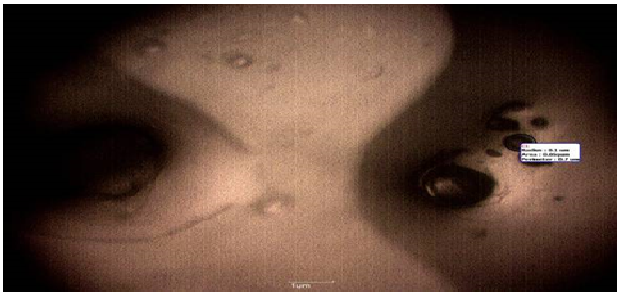
Thus suggesting the amount of drug that would be available at the site of absorption. This parameter is dependent on the process of preparation, physicochemical properties of drug, and formulation variables [11]. It is also highly influenced by type of polymer, polymer concentration and solvent used to dissolve the drug and polymer [6]. Microspheres formulated with 3 g of Eudragit® RL100 entrapped the highest amount of insulin compared with the rest of the formulations.

**Table 3:** Thermal properties of the formulations

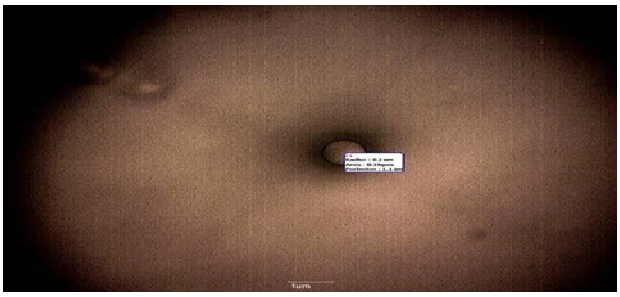
| Formulation code | Melting point (°C) | Enthalpy (mW/mg) |
|---|---|---|
| **Insulin** | 125 | -132 |
| **$U_0$** | 62.7 (78.2) | - 4.216 (-4.131) |
| **$U_1$** | 63.6 | -4.777 |
| **$U_2$** | 64.2 (85.6) | - 8.821 (-7.662) |
| **$U_3$** | 63.9 (81.6) | - 3.360 (- 2.561) |

**Keys:** $U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100 while $U_0$ is the unloaded microspheres

The particle size distribution of the microspheres is presented in Table 2. The mean particle size (n = 30) of insulin-loaded microspheres ranged from 14.20 ± 0.30 µm to 19.80 ± 0.60 µm whereas the mean particle size of unloaded microspheres batch was 13.50 ± 0.90 µm. Thus plain microspheres had the smallest mean particle size while insulin-loaded microspheres prepared with highest amount of Eudragit® RL100 (300 mg) possessed the largest mean particle size. The photomicrographs of the microspheres are depicted in Fig. 2. Generally, discrete, spherical, brownish and free flowing microspheres were obtained. The sizes of the microspheres were all within the micrometer range, indicating that the production process was able to achieve the intended end-point, consistent with previous reports [6, 11]. It would appear that the average size of the microspheres increased with an increase in the proportion of the polymer employed. This could be attributed to the fact that greater amounts of the polymeric materials formed thicker coatings around the drug particles leading to increased average size of the microspheres. Particle size of microspheres is an important parameter, since it affects drug release and pharmacokinetics [8]. For microspheres engineered for parenteral administration, large particles would find it difficult to pass through the syringe. However, the microspheres evaluated in this study are intended for oral administration and particle size will influence only the rate of drug release and subsequent pharmacokinetics.



**(2a)**



**(2b)**

**(2c)**

**Figure 2:** Photomicrographs of insulin-loaded microspheres containing various amounts of Eudragit® RL100 (batch $U_0$) (a) 2 g (batch $U_1$), (b) 3 g (batch $U_2$), (c) 4 g (batch $U_3$).
**Keys:** $U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100.

The results of the mucoadhesion of the microspheres to cow everted intestinal tissue as evaluated in SIF are presented in Table 2. It is evident from the table that the microspheres formulated showed good mucoadhesive properties and exhibited percentage mucoadhesion as high as 75.0 % for unloaded microspheres and between 60.29 and 80.0 % for insulin-loaded microspheres. The mucoadhesive property of unloaded microspheres was comparable to that of drug-loaded microspheres. Although there was no particular order of mucoadhesiveness with respect to the proportion of polymer employed in the study,

the order of the mucoadhesiveness of the microspheres batches is: $U_2 > U_0 > U_1 > U_3$. Thus, batch $U_2$ prepared with 300 mg of Eudragit® RL 100 had the highest percentage mucoadhesion. The high percentage mucoadhesion of the different batches of the microspheres in SIF signifies high mucoadhesive property in intestinal conditions where insulin absorption takes place [2-4]. This shows that the insulin-loaded microspheres will have a prolonged release and thereby enhance bioavailability of insulin. The quaternary ammonium groups of Eudragit® RL100 confer a positive zeta potential to microspheres which can interact with the negative charges of intestinal mucus (due to the presence of sialic acid in its composition). These interactions could be responsible for mucoadhesion of microspheres on the surface of the intestinal barrier, allowing a closer intimacy of contact between drug and mucous membrane at the absorption sites, thus enhancing the permeability as well as reducing the local degradation of the drug [10, 16, 41]. In other words, the mucoadhesive results indicate that the microspheres may be preferable as carriers for drugs such as insulin targeted to have drug residence time in the small intestine. By implication, the microspheres formulations may be a novel oral controlled drug delivery system for the delivery of insulin to the intestine owing to its high mucoadhesiveness in SIF.

The response curves obtained by plotting the blood glucose reduction levels versus time are depicted in Fig. 3. In some of the animals, the blood glucose levels were higher than the initial levels within the first hour of administration. The orally administered insulin solution, distilled water and unloaded microspheres ($U_0$) served as negative controls whereas subcutaneously administered insulin served as positive control. Distilled water and orally administered insulin solution generally did not cause any significant reduction in blood glucose levels. Orally administered insulin solution and distilled water resulted in a slight fall in the blood glucose level within 1 and 2 h respectively of administration, and increased consistently thereafter. Subcutaneously administered insulin solution caused consistent fall in the blood glucose level starting from 30 min post-administration. The three batches of the insulin-loaded microspheres formulations caused a reduction in the blood glucose levels within 12 h of the study. The percentage blood glucose reduction for the subcutaneously (sc) administered insulin was significantly ($p \leq 0.05$) higher than for all the formulations. The effectiveness of the oral insulin microspheres prepared with Eudragit® RL 100 was assessed based on its potential in causing blood glucose reduction in alloxan-induced diabetic rats. The administration of equal doses of insulin, subcutaneously, orally and in the formulations was to give direct comparison of the dose response in the study. In some of the animals the blood glucose levels were higher than the initial levels within the first hour of administration which could be attributed to the stress associated with drug administration [40]. A more efficient reduction in the blood glucose level could have masked this initial increase in other animals. Eudragit® RL 100 is a low water permeable, cationic, non-biodegradable polymer commonly used for enteric coating of tablets and for the preparation of controlled-release dosage forms and represents a good material for the dispersion of drugs [23]. High blood glucose reduction resulting from insulin-loaded microspheres may be attributed to insulin protection or absorption within the GIT. It is quite improbable for insulin absorption to occur in the stomach, thus the reduction in the blood glucose after oral administration of insulin solution could be due to some of the insulin solution reaching the intestine since high doses of insulin solution were administered to the rats. Blood glucose reduction occurred within 30 min of oral administration in some samples. This could be attributed to such factors as early gastric emptying of the drug from the stomach into the small intestine and the effective mucoadhesiveness of the polymer matrix (Eudragit® RL 100) that was efficient in adhering the microspheres to the gastric mucosa and protecting the insulin from degradation [17, 21]. It was helpful to protect insulin activity against the enzymatic attack in harsh environment of stomach and intestine. The modification of the microspheres and suitable size of the microspheres made them easily adhere on the intestinal mucosa and transfer into the blood circulation, consistent with similar reports on oral insulin delivery [9 - 11]. Overall, the results suggest that insulin-loaded Eudragit® RL 100 entrapped microspheres could be orally administrated for management of diabetes.

## CONCLUSIONS

The results obtained from our studies have demonstrated the effectiveness of the formulations as a carrier system for oral insulin delivery. All the insulin-loaded microspheres

produced blood glucose lowering effect after 12 h. This indicates that oral delivery of insulin for effective control of blood glucose is indeed possible using Eudragit® RL100 entrapped microspheres.

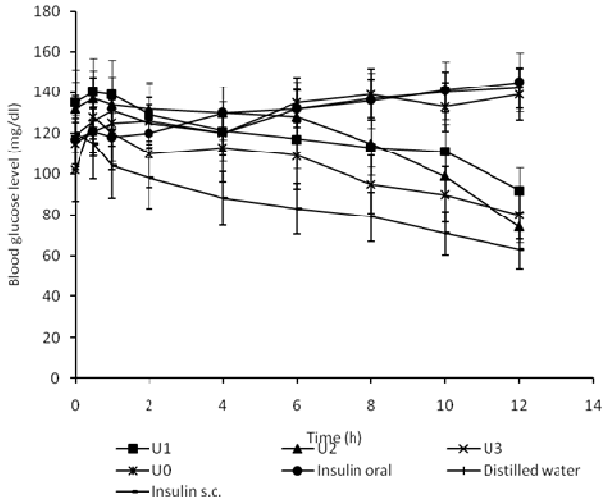



**Figure 3:** Profiles of reduction in blood glucose levels produced by the formulations.
**Keys:** $U_1$, $U_2$ and $U_3$ are insulin-loaded microspheres containing 2, 3 and 4 g of Eudragit® RL 100; $U_0$ is unloaded microspheres

## REFERENCES


[1] Aguwa CN. Therapeutic Basis of Clinical Pharmacy in the Tropics, Aguwa CN (ed.) SNAAP Press Ltd., Enugu, Nigeria, 2004, pp. 189-192.

[2] Kinesh VP, Neelam DP, Punit BP, Bhavesh SB, Pragna KS. Novel approahces for oral delivery of insulin and current status of oral insulin products. Int J Pharm Sci Nanotechnol. 2010; 3: 1057 – 1064.

[3] Marias E, Hamman J, du Plessis L, Lemmer R, Steenekamp J. Eudragit ®L100/N-Trimethylchitosan chloride microspheres for oral insulin delivery. Molecules. 2013; 18: 6734 – 6747.

[4] Gowthamarajan K, Kulkami GT. Oral insulin – fact or fiction – Resonance. 2003; 55: 38-43.

[5] Kumria R, Goomber G. Emerging trends in insulin delivery: Buccal route. J Diabetol. 2011; 2: 1–9.

[6] Timmy SA, Victor SP, Sharma CP, Valsala KJ. Betacyclodextrin complexed insulin-loaded alginate microspheres – oral delivery system. Trends Biomater Artif Organs. 2002; 15: 48–53.

[7] Morishita M, Peppas NA. Is the oral route possible for peptide and protein drug delivery? Drug Discov Today. 2006; 11: 905–910.

[8] Yan P, Jun-Min Z, Hui-Ying Z, Ying-Jian L, Hui X, Gang W. Relationship between drug effects and particle size of insulin-loaded bioadhesive microspheres. Acta Pharmacol Sin. 2002; 23: 1051–1056.

[9] Celek A, Celebi N, Tirnaksiz F, Tay A. A lecithin-based microemulsion of Rh-insulin with aproprotin for oral administration: Investigation of hypoglycaemic effects in non-diabetic and STZ-induced diabetic rats. Int J Pharm. 2005; 298: 176-185.

[10] Eaimtrakarn S, Ramaprasad YV, Ohno T, Konishi T, Yoshikawa Y, Shibata N. Absorption-enhancing effect of labrasol on the intestinal absorption of insulin in rats. J Drug Target. 2002; 10: 255-260.

[11] Zhang Y, Wei W, Lv P, Wang L, Ma G. Preparation and evaluation of alginate-chitosan microspheres for oral delivery of insulin. Eur J Pharm Biopharm. 2011; 77: 11–19.

[12] Heinemann L, Pfutzner A, Heise T. Alternative routes of administration as an approach to improve insulin therapy: update on dermal, oral, nasal and pulmonary insulin delivery. Curr Pharm Des Sep. 2001; 7: 1327-1351.

[13] Nakamura K, Murray RJ, Joseph JI, Peppas NA, Morishita M, Lowman AM. Oral insulin delivery using P(MAA-g-EG) hydrogels: effects of network morphology on insulin delivery characteristics. J Control Rel. 2004; 95: 589-599.

[14] Sajeesh S, Sharma CP. Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. Int J Pharm. 2006; 325: 147-154.

[15] Akhter DT, Nijhu RS. Diabetes mellitus: A journey of insulin. Int Curr Pharm J, 2012; 1: 32–42.

[16] Evans M, Schumm-Draeger PM, Vora J, King AB. A review of modern insulin analogue pharmacokinetic and pharmacodynamic profiles in type 2 diabetes: improvements and limitations. Diabet Obes Metab. 2011; 13: 677–684.

[17] Jain D, Panda AK, Majumdar DK. Eudragit® S 100 entrapped insulin microspheres for oral delivery. AAPS Pharm Sci Tech. 2005; 6: 1-27.

[18] Lamprecht A, Koenig P, Ubrich N, Maincent P, Neumann D. Low molecular weight heparin nanoparticles; mucoadhesion and

behaviour in Caco-2 cells. Nanotechnol. 2006; 17: 3673-3680.

[19] Vaghani SS, Jivani NP, Vasanti S, Satish CS, Patel MM. Preparation and characterization of 5-FU loaded microspheres of Eudragit® and ethylcellulose. Acta Pharm Sci. 2010; 52: 391-399.

[20] Paharia A, Yadar AK, Rai G, Jain SK, Pacholi SS, Agrawal GP. Eudragit®-coated pectin microspheres of 5-Fluorouracil for colon targeting. AAPS Pharm Sci Tech. 2007; 8, E1-E7.

[21] Okada H, Toguchi H. Biodegradable microspheres in drug delivery. Crit Rev Ther Drug Carrier Syst. 1995; 12: 1-99.

[22] Kishori LD, Nilima AT, Paraag SG. Formulation and development of tinidazole microspheres for colon targeted drug delivery system. J Pharm Res. 2013; 5: 158-160.

[23] Haznedar S, Dortunc B. Preparation and *in vitro* evaluation of Eudragit® microspheres containing acetazolamde. Int J Pharm. 2004; 269: 131-140..

[24] Hogan JE. Film–coating materials and their properties. In: Pharmaceutical coating technology, Cole G. (ed.) Special Ed., Informa Healthcare, New York, 2008, pp. 7-19.

[25] Pamnani D. Reality check on oral insulin. Pharma Express. 2008; 3: 16-31.

[26] Rosa GD, Iommeli R, Rotonda MIL, Mitro A, Quaglia F. Influence of the co-encapsulation of different non-ionic surfactants on the properties of PLGA insulin-loaded microspheres. J Control Rel. 2000; 69: 283-295.

[27] Singh S, Patel D, Patel NR, Kumar K, Gautam MK. Insulin oral delivery may be possible. Int J Pharm Prof Res. 2010; 1(1): 46–51.

[28] Whitehead K, Shen Z, Mitragotri S. Oral delivery of macromolecules using intestinal patches: Applications for insulin delivery. J Control Rel. 2004; 98: 37-45.

[29] Zhang Y, Wu X, Meng L, Zhang Y, Ai R, Qi N, He H, Xu H, Tang X. Thiolated Eudragit nanoparticles for oral insulin delivery: Preparation, characterization and *in vivo* evaluation. Int J Pharm. 2012; 436: 341–350.

[30] Tuesca A, Lowman A. The oral delivery of insulin using protein conjugates in complexation hydrogels. Poster presentation, Biomaterials and drug delivery laboratory, Drexel University, Philadelphia, Pennsylvania, USA, 2006.

[31] Caliceti P, Veronese EM. Improvement of the physicochemical and biopharmaceutical properties of insulin by poly (ethylene glycol) conjugation. STP Pharm Sci. 1999; 9: 107 – 113.

[32] Cui FD, Shi K, Zhang L. Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery: Preparation, *in vitro* characterization and *in vivo* evaluation. J Control Rel. 2006; 114: 242–250.

[33] Cui FD. Preparation of insulin-loaded PLGA-Hp55 nanoparticles for oral delivery. J Pharm Sci. 2007; 96: 421-427.

[34] Damge C, Hillaire-Buys D, Peuche R, Hoeltzel A, Micheal C, Ribes G. Effect of orally administered insulin nanocapsules in normal and diabetic dogs. Diabetes Nutr Metab. 1995; 8: 3–9.

[35] Damge C, Maincent P, Ubrich N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Rel. 2007; 117: 163-170.

[36] Kavimandan NJ. Synthesis and characterization of insulin-transferrin conjugates. Bioconjug Chem. 2006; 17: 1376-1384.

[37] Krauland AH, Guggi D, Bernkop-Schnurch A. Oral insulin delivery: The potential of thiolated chitosan-insulin tablets on non-diabetic rats. J Control Rel. 2004; 95: 547-55.

[38] Lin YH. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. Biomacromolecules. 2007; 8: 146-152.

[39] Ofokansi KC, Adikwu MU. Formulation and evaluation of microspheres based on gelatin-mucin admixtures for the rectal delivery of cefuroxime sodium. Trop J Pharm Res. 2007; 6: 825-832.

[40] Builders PF, Kunle OO, Okpaku LC, Builders MI, Attama AA, Adikwu MU. Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. Eur J Pharm Biopharm. 2008; 70: 777–783.

[41] Iwanaga KS, Ono S, Naroika K, Morimoto K, Kakemi M, Yamashita S, Nango M, Oku N. Oral delivery of insulin by using surface coating liposomes for improvement of insulin in GI tract. Int J Pharm. 1997; 157: 73–80.