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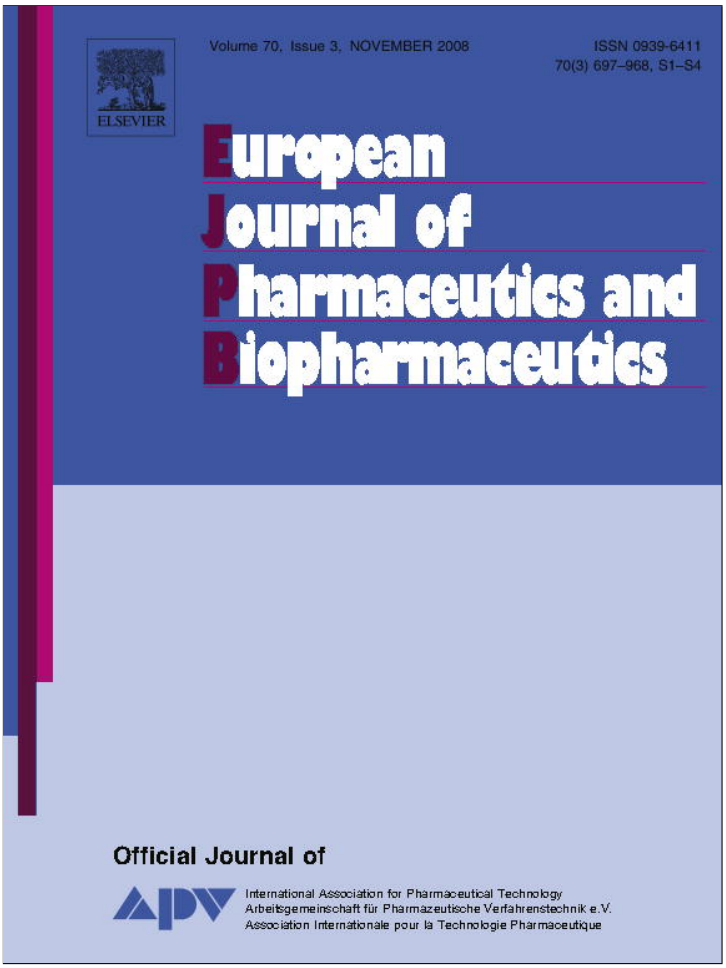

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European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 777–783

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin

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ARTICLE INFO

Article history:
Received 11 December 2007
Accepted in revised form 19 June 2008
Available online 3 July 2008

Keywords:
Mucin
Sodium alginate
Microparticles
Insulin release
Oral administration
Blood glucose reduction

ABSTRACT

Effective oral insulin delivery remains a challenge to the pharmaceutical industry. In this study, insulin-loaded microparticles for oral delivery were prepared with mucin and sodium alginate combined at different ratios using a novel method based on polymer coacervation and diffusion filling. Some physical characteristics of the various insulin-loaded microparticles such as particle size, morphology and compressibility indices were determined. The microparticles were filled into hard gelatin capsules and the *in vitro* insulin release as well as the blood glucose reduction after oral administration to diabetic rabbits were determined. The microparticles formed were generally multi-particulate, discrete and free flowing. Before insulin loading, microparticles were round and smooth, becoming fluffier, less spherical and larger with rough and pitted surface after insulin loading. The insulin content of the microparticles increased with increase in their sodium alginate content. The various insulin-loaded microparticles prepared with the mucinated sodium alginate when encapsulated exhibited lag time before insulin release. The time taken to reach maximum insulin release from the various formulations varied with the mucin–sodium alginate ratio mix. The mean dissolution time of insulin from the microparticles prepared with sodium alginate, mucin, sodium alginate: mucin ratios of 1:1, 3:1 and 1:3 was 11.21 ± 0.75, 3.3 ± 0.42, 6.69 ± 023, 8.52 ± 0.95 and 3.48 ± 0.65 (min.), respectively. The percentage blood glucose reduction for the subcutaneously administered insulin was significantly ($p < 0.001$) higher than for the formulations. The blood glucose reduction effect produced by the orally administered insulin-loaded microparticles prepared with three parts of sodium alginate and one part of mucin after 5 h was, however, equal to that produced by the subcutaneously administered insulin solution, an indication that it is an effective alternative for the delivery of insulin.

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1. Introduction

The peptide insulin, which is used for the management of diabetes mellitus, is administered parenterally by subcutaneous injection [1], which results in low level of compliance due to pain. The oral route is considered to be the most acceptable and convenient route of drug administration for chronic therapy. However, during insulin transit through the gastrointestinal tract (GIT) it is chemically and enzymatically inactivated due to high acidity and proteolytic enzyme activity [2]. Several attempts have been explored to optimize insulin stability and absorption within the GIT [3–6]. The successful production of an effective oral insulin formulation will depend on the development of an appropriate functional polymer that will protect insulin from the degradative effect of enzymes and pH of the GIT [7]. One possible method of preparation of such new carrier materials is by hybridization or crosslinking of polymers with different but desirable properties. This will be most effective if the hybrid polymer is pH responsive and with good protective characteristics [8].

Mucins are high molecular weight glycosylated proteins, believed to be the major structure-forming component of mucus [9] and responsible for the cohesive and visco-elastic nature of mucus gel [10]. Pure mucin is the glycoprotein part of the mucus devoid of water, free proteins, minerals and lipids [9]. Apart from acting as protectants and lubricants, mucins are known to be the substrate on which mucoadhesive polymers attach [11], thus the

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doi:10.1016/j.ejpb.2008.06.021

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interest in hybridizing them with other polymers [12]. Sodium alginate is a biocompatible biodegradable hydrophilic polymer obtained from the brown seaweed. It has been used in micro- and nano-particle formulations for controlled delivery of some bioactive materials due to its gelation, bioadhesive and pH responsive properties [13,14]. Cellulose acetate phthalate is a cellulose polymer in which about half the hydroxyl groups are acetylated and about a quarter are esterified with one of two acid groups being phthalic acid while the other is free. It is a biocompatible polymer that is used in pharmaceutical formulations at concentrations of 0.5–9% as an enteric coating material or matrix binder for tablets and capsules. Film coating with cellulose acetate phthalate imparts prolonged resistance to dissolution when in contact with the strongly acidic gastric fluid, but dissolves in the mildly acidic or neutral intestinal environment [14].

This study was designed to formulate insulin-loaded microparticles for oral insulin delivery with mucin–sodium alginate hybrids using a novel method based on coarcervation and enteric film coating processes under controlled temperature. The enteric-coated mucinated sodium alginate microparticles were loaded with insulin by diffusion filling. Evidence of the effectiveness of the oral insulin delivery in terms of reduction of initial blood glucose level was assessed on diabetic rabbits.

2. Materials and methods

2.1. Materials

Sodium alginate (Mwt. 260,000), porcine mucin (Type III, Mwt. ≈ 125,000,000) and alloxan were all obtained from Sigma (Sigma Chemical company, USA), cellulose acetate phthalate (Mwt. ≈ 60,000, Sartorius AG, Germany), potassium dihydrogen phosphate (May & Baker Ltd., Dagenham, England), sodium hydroxide (BDH, England), GlucoPlus (GlucoPlus Inc., Canada) and insulin (Novo Nordisk A/S, Denmark) were used as procured without further treatment.

2.2. Preparation of microparticles

The ratios of mucin and sodium alginate used for preparing the empty microparticles are shown in Table 1.

Ten gram-quantities of mucin–sodium alginate ratio mixtures as in Table 1 were dispersed in distilled water to produce a 10% w/v homogeneous dispersion. Each dispersion was transferred into a 250 ml beaker containing 150 ml of liquid paraffin. The mix was then dispersed in the liquid paraffin using a Kenwood mixer (Kenwood Ltd., USA) set at a stirring speed of 350 rpm. The mucin–sodium alginate mixture in liquid paraffin was then slowly poured into a 1000 ml beaker containing 500 ml of acetone maintained at −30 °C with continued mixing at a lower speed (200 rpm), for 10 min. The beaker was immersed in an ice jar to minimize increase in temperature.

The microparticles formed were then recovered by filtration using a filter paper (Whatman, USA). The microparticles were washed five times with 50 ml quantities of acetone at −30 °C to remove any trace of the liquid paraffin.

Table 1
Ratios of mucin and sodium alginate used for preparing empty microparticles

Formulation	Mucin	Sodium alginate
A	1	0
B	0	1
C	1	1
D	3	1
E	1	3

2.3. Coating of microparticles with cellulose acetate phthalate

A 100 ml-volume of a 1.5% w/v solution of cellulose acetate phthalate in acetone was prepared and stored at −30 °C for 24 h. Five grams of the microparticles was added to 30 ml of the chilled cellulose acetate phthalate solution in acetone in a large test tube and mixed using a vortex mixer (Vortex Genie 2. Bohemia, USA) for 5 min at No. 2 setting. The coated microparticles were recovered by filtration with a filter paper (Whatman, USA) and dried with a flush of cold air (10 °C). The coating process was done twice. The microparticles were placed in a Petri dish inside a desiccator for 24 h at 5 °C and finally stored in an airtight screw capped bottle at 5 °C until used.

2.4. Insulin loading of the microparticles

Insulin was loaded into the various microparticles by the diffusion loading method [15,16]. A 1 g-quantity of the coated microparticles produced as in Table 1 was placed in a 20 ml beaker, 5 ml quantities of the insulin solution (pH 7.3 and 100 IU/ml) adjusted to pH 6 with few drops of 0.01 N HCl was then introduced and allowed to stand for 2 h at 5 °C. The microparticles were rinsed with 15 ml of 0.01 N HCl followed by 15 ml of distilled water. The hydrated microparticles were then freeze-dried for 12 h. The freeze-dried material was passed through sieve No. 20 (USA Standard testing sieve, USA) and stored in airtight screw capped bottles maintained at 5 °C until used.

2.5. Characterization of microparticles

The particle size and size distribution of the microparticles prepared with mucin and the various mixtures with sodium alginate before and after insulin loading were determined using the sieve method. Sieves (Endecott, UK) with different mesh sizes (75–1000 μm) were arranged in the descending order with the collector pan placed at the bottom. A 10 g quantity of the microparticles was poured on the top sieve and shaken for 5 min. The weight of the particles retained on each sieve was then determined and plotted against the mean particle size [17].

The microparticle bulk and tapped densities and particle size before and after insulin loading were determined. The compressibility indices of the particles were also evaluated from the bulk and tapped density using Eq. (1) [18].

$$C = (1 - v/v_0 \times 100), \quad (1)$$

where C is percentage compressibility or Carr's index, v is tapped volume and v_o is the bulk volume.

2.6. Morphology of microparticles

The scanning electron micrographs (SEM) of the various formulations of microparticles were obtained. The samples were prepared by goldplating the particles. Imaging of the formulations was carried out on a FEI Quanta 400 electron scanning microscope (FEI Company, Oregun, USA) at a magnification of ×250.

2.7. Quantitative determination of insulin

The insulin content of the microparticles was determined using a high performance liquid chromatography (HPLC). The system consisted of an Agilent 1100 series programmable separating module, quaternary pump G1311A (Agilent technology, USA), an auto degasser G1322A, and a variable wavelength detector G1314A. The column was a reverse phase ODS (C-18, 5 μm 4.6 × 250 mm, Supercosol, USA) equipped with a guard. The mobile phase consisted of acetonitrile and water (10:90), perchloric acid was used

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to adjust the pH to 3. The flow was set at 0.8 ml/min. The chromatograms were recorded at 280 nm.

2.8. Insulin loading efficiency

A 10 mg quantity of microparticles was dispersed in 10 ml of phosphate buffer (pH 7.4). The dispersion was allowed to stand for 2 h after which it was mixed in a vortex mixer for 5 min and then centrifuged at 4000 rpm for 10 min. The amount of insulin contained in the various microparticulate formulation samples was determined using HPLC. The insulin loading efficiency was then determined using Eq. (2) [16].

$$\mathrm{ILE} = (\mathrm{AD}/\mathrm{TD}) \times 100, \quad (2)$$

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

2.9. In vitro release of insulin from microparticles

The *in vitro* release profiles of the insulin-loaded microparticles was determined [19]. A 100 mg quantity of the insulin-loaded microparticles was filled into hard gelatin capsules. Each capsule was then placed in a 250 ml beaker containing 150 ml of phosphate citrate buffer solution (pH 2.2), agitation of the fluid system (100 rpm) was done with a magnetic stirrer. At predetermined time intervals, 0.5 ml samples were withdrawn and replaced with phosphate citrate buffer solution. After 1 h the pH of the dissolution medium was changed to 6.5 by the addition of 0.1 N sodium hydroxide and further sampling continued for another 3 h. The temperature of the dissolution system and the replacement fluid were maintained at 37 ± 0.5 °C [19]. The insulin content of the withdrawn samples was determined using the HPLC quantitative method. The release of insulin per unit time was fitted into Eq. (3) [20].

$$M_t/M_\infty = Kt^n. \quad (3)$$

Here M_t/M_∞ is the fraction of insulin released at time t; K is a constant incorporating the properties of the polymeric system and n is a kinetic constant that is used to characterize transport mechanism governing the drug release from the matrix. The values of n and K were evaluated from the plot of Ln (M_t/M_∞) vs. Ln t, *where* n and K are the slope and intercept at the y-axis, respectively [21]. The fraction of insulin released at time t (10–120 min) only was considered. The rate of insulin release from the different formulations was determined by evaluating the mean dissolution time (MDT) using Eq. (4), where K and n have the same meaning as in Eq. (3) [20].

$$\mathrm{MDT} = (n/n+1)K^{-(1/n)} \quad (4)$$

2.10. In vivo antihyperglycaemic assessment

2.10.1. Induction of diabetes

Rabbits weighing between 1.8 kg and 2.5 kg were obtained from the NIPRD animal utility house. The rabbits were all kept in standard and conditioned animal cages and left for one week to acclimatize to the new cages. They were fed with standard rations. Diabetes was induced by intravenous injection of alloxan at a dose of 120 mg/kg dissolved in normal saline through the marginal ear vein [22]. Diabetes was allowed to develop and stabilize for 5 days during which time the animals were allowed food and water *ad libitum* [23]. Before the commencement of the experimental work, the protocol on the use of animal was approved by the Ethical Committee of National Institute for Pharmaceutical Research and Development, Abuja.

2.10.2. Protocol for administration of insulin microparticles

The 32 diabetic rabbits were randomly divided into eight groups of four with each group housed in a separate cage. The different formulations of the insulin-loaded microparticles were filled into hard gelatin capsules, with each capsule containing microparticles equivalent to insulin dose of 50 IU/kg body weight for each animal. The capsules were administered orally to the animals in one group according to their weight. The second received insulin solution (50 IU/kg) orally; the third received insulin solution (50 IU/kg) subcutaneously, while the fourth received distilled water orally [24].

2.10.3. Assessment of the hypoglycaemic effect of oral insulin on diabetic rabbits

Prior to commencement of the studies the animals were fasted for 16 h and blood glucose concentration was determined using a glucometer (GlucoPlus, GP0012786, Canada) and blood glucose tests strips with blood samples collected from the ear vein. Blood glucose values of 120 mg/dl and above were considered diabetic [23]. The diabetic rabbits were then treated according to the protocol and blood glucose concentrations determined at predetermined times up to 8 h.

3. Results and discussion

The formulation concept of insulin-loaded microparticles prepared with physical crosslinks of mucin and sodium alginate was aimed at enhancing the absorption of insulin from the GIT, by providing a protective environment for insulin and improved mucoadhesion of the insulin-loaded microparticles. The process of formulation of the insulin-loaded microparticles is based on temperature-controlled solvent-induced coacervation technique. It was observed that while microparticles were formed at −30 °C, none was obtained at temperatures above −10 °C. The low temperature probably stabilized the microparticles by the formation of crystallites through hydrogen bonds among amine, amide, carboxylic and hydroxyl groups in the mucin and sodium alginate polymer networks [25]. These (the crystallites) then act as physical crosslink sites. The low temperature was also useful in minimizing the incompatibilities of proteins with the organic solvents so that mucin is not denatured by acetone. Hydrophobic interactions between the functional groups in sodium alginate and mucin may also occur through inter and intra molecular hydrogen bonding. This will shield ionizable polar groups and prevent their interactions with water molecules, thereby contributing to reduction in polymer swelling. Thus, reducing insulin release from the hybrid polymer matrix [26]. Insulin may form non-covalent complexes with the polymers via protein–protein or protein–polysaccharide interactions [16]. The formation of such complexes and the entrapment of insulin within the hybrid polymer matrix will contribute to the stability of insulin in the microparticles.

Discrete, free flowing microparticles were successfully prepared with mucin, sodium alginate and mucin–sodium alginate mixtures (Table 1). The microparticles formed by the coacervation process was due to the phase separation of the hydrogels resulting from their relative insolubility in acetone. Acetone used at −30 °C will increase the degree of aggregation of the hydrogel dispersions [27] and minimize the denaturation of mucin [26]. Although, denaturation can be minimized by precipitating at below 0 °C, mixing protein dispersions with acetone is characterized by temperature rise due to negative heat change of hydration of the solvent, thus the need to maintain acetone at −30 °C. Working at such low temperature was maintained to ensure that mucin does not lose its viscoelastic integrity as the maximum optimum storage temperature for porcin mucin (type III) is 8 °C. Temperatures higher than the optimum storage temperature and the presence of acetone will

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accelerate mucin denaturation. Denaturation of mucin would result from the breaking of the bonds holding the protein and oligosaccharides in their native conformation, and the release of associated solvent molecules. Thus, the destabilization of the glycoprotein molecular structure resulting in the loss of its functional properties such as its viscoelasticity and mucoadhesiveness [26]. Despite working at low temperature, there was no direct exposure of insulin to acetone because of the potential risk of denaturation.

There was a tendency for the microparticles to agglomerate in the presence of liquid paraffin, hence the need to ensure thorough removal of all traces of liquid paraffin in the microparticles. Apart from precipitating and washing the microparticles with acetone maintained at −30 °C, drying of the harvested microparticles with a flush of cold air was also to prevent a possible denaturation of mucin by residual acetone which could rapidly occur at higher temperatures [26]. Insulin loading of the enteric-coated microparticles by diffusion filling and freeze-drying, and storage at 5 °C were processes employed to maintain stability.

3.1. Photomicrograph

Representative photomicrographs of the microparticles prepared with mucin and sodium alginate before and after insulin loading are presented in Figs. 1 and 2. The surface of the microparticles before insulin loading was generally rounded and smooth; however, after the diffusion loading, freeze-drying and screening processes the microparticles lost the round shapes and their surface became rough and pitted. This was due to the structural deformation of the microparticles due to the freeze-drying and sieve screening processes.

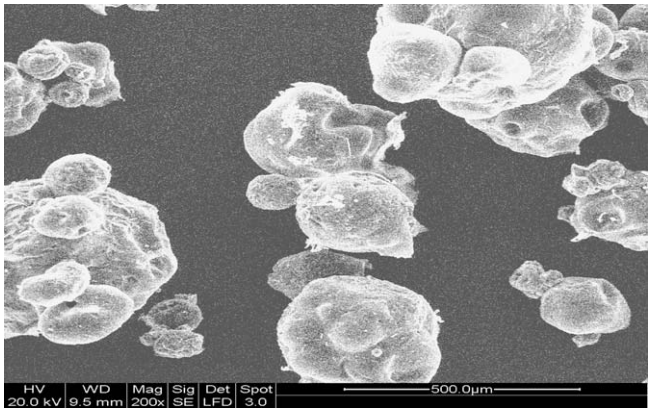

Fig. 1. Microparticles prepared with one part of mucin and one part of sodium alginate before insulin loading X 200.

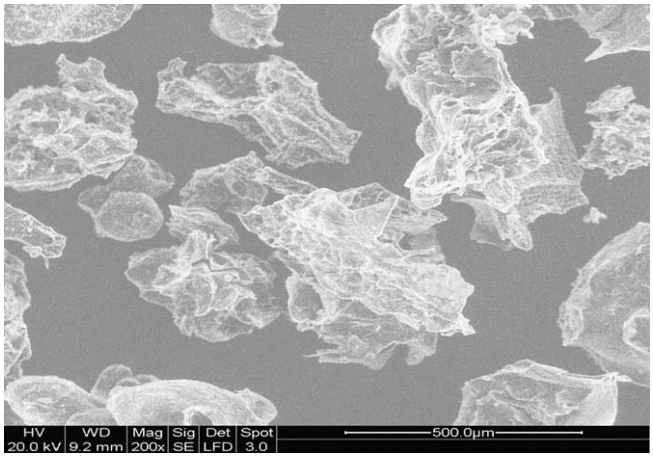

Fig. 2. Microparticles prepared with one part of mucin and one part of sodium alginate after insulin loading X 200.

3.2. Physical properties of the insulin-loaded/-unloaded microcapsules

The bulk and tapped densities as well as the compressibility indices of the microparticles before and after insulin loading are presented in Table 2. The polymer type and ratio mixtures used for the preparation of the microparticles as well as the freeze-drying and sieve screening processes affected the bulk and tapped densities and compressibility indices of the formulations. The bulk and tapped densities of the microparticles before insulin loading were generally higher than after loading. This could be due to changes in the particle shape and sizes resulting from the structural changes in the morphology of the microparticles that occurred during processing. The insulin-loaded microparticles are fluffier and less spherical than the microparticles before insulin loading, freeze-drying and sieve screening as shown in Fig. 1.

The compressibility indices of the insulin-loaded microparticles were generally higher than that of the unloaded microparticles except for the microparticles prepared with sodium alginate alone. The difference in compressibility indices between the microparticles before and after insulin loading was significant for each ratio mix ($p < 0.01$). This indicates that the processes involved in insulin loading affected the flow of the microparticles. The discrepancy observed for microparticles prepared with sodium alginate may be due to the minimal changes in the structure of the microparticles during the freeze-drying process. Thus, the differences in the bulk and tapped densities, and the compressibility indices of the microparticles before and after insulin loading processes are due to structural changes that occurred during the insulin diffusion filling, freeze-drying and the sieve screening processes (see Fig. 3).

3.3. Particle size and particle size distribution of microcapsules

The particle size and size distribution of microparticles prepared by this dispersion and coacervation process were influenced by such factors as speed of the dispersion of the polymer in the oil and viscosity of the polymer or polymer mix [28]. However, since the mixing speed was constant in all the formulations any variation observed would be mainly due to viscosity.

The average particle sizes for all the insulin-loaded microparticle formulations were higher than the unloaded microparticles. This could result from the structural reorientation in the particles due to the clumping that resulted from freeze-drying and the sieve screening operation or interactions between mucin and sodium alginate.

Before insulin loading, the average particle size for mucin, sodium alginate and sodium alginate–mucin 1:3 ratio mixture was the same (220 μm), while those of sodium alginate–mucin ratio mixtures at 1:1 and 3:1 were the same (600 μm). However, the average particle sizes of the microparticulate formulations with the various ratio mixtures after insulin loading were sodium alginate 310 μm; mucin 230 μm; sodium alginate–mucin (3:1) 260 μm; and sodium alginate–mucin (1:3) 680 μm (see Fig. 4).

3.4. Insulin loading efficiency

Fig. 5 shows the insulin loading efficiency of the enteric-coated microparticles formulated with the various ratio mixtures of sodium alginate and mucin. During the insulin loading process, the pH of the insulin solution was adjusted from 7.3 to pH 6 to minimize the erosion of the cellulose acetate phthalate coat. The thin cellulose acetate coat, at pH 6, formed a thin gel layer that

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Table 2
Bulk and tapped densities, and compressibility index of microparticle before and after insulin loading

	M	M*	Alg	Alg*	M1 : Alg1	M1* : Alg 1	M3 : Alg1	M3* : Alg1	M1 : Alg3	M1* : Alg3
Bulk density (g/cm^3)	0.379 ± 0.022	0.214 ± 0.05	0.299 ± 0.006	0.217 ± 0.001	0.343 ± 0.001	0.206 ± 0.001	0.342 ± 0.002	0.162 ± 0.013	0.412 ± 0.01	0.217 ± 0.01
Tapped density (g/cm^3)	0.474 ± 0.04	0.287 ± 0.05	0.42 ± 0.004	0.41 ± 0.002	0.455 ± 0.001	0.266 ± 0.001	0.457 ± 0.003	0.231 ± 0.001	0.505 ± 0.03	0.298 ± 0.01
Carr index (%)	20 ± 0.04	26.07 ± 0.03	28.81 ± 0.02	22.6 ± 0.34	24.62 ± 0.23	29.13 ± 0.11	25.16 ± 0.61	29.87 ± 0.1	18.42 ± 0.02	27.18 ± 0.12

M, Mucin.
Alg, Sodium alginate.
*After insulin loading.

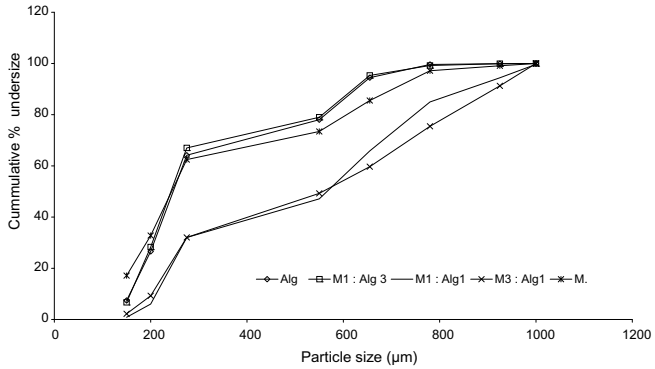

Fig. 3. Particle size distribution curve for microparticles prepared with mucin and sodium alginate mixtures at various ratios: before insulin loading.

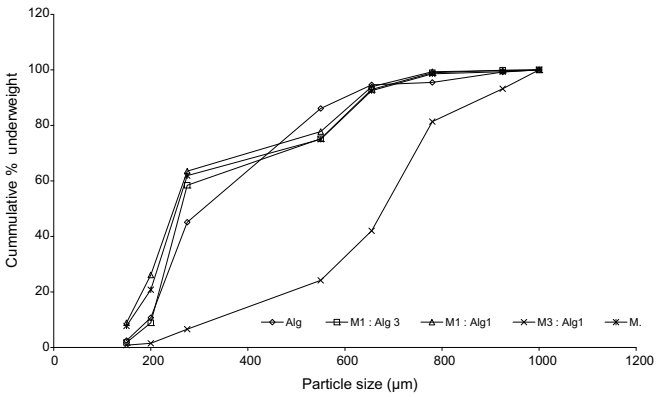

Fig. 4. Particle size distribution curve for microparticles prepared with mucin and sodium alginate mixtures at various ratios: after insulin loading.

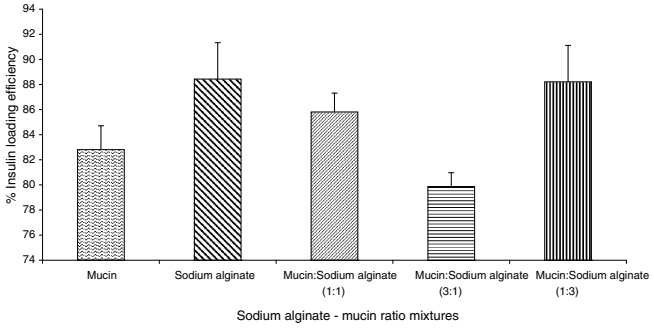

Fig. 5. Insulin loading efficiency of microparticles prepared with sodium alginate–mucin ratio mixtures.

permitted the slow ingress of the insulin solution into the micro-matrix while still maintaining the structural integrity of the microparticles. Coating the microparticles with cellulose acetate phthalate was aimed at maintaining the structural integrity of the particles during insulin diffusion filling and to target the release of insulin in the small intestine. The relatively high concentration of insulin solution (100 IU/ml) used for incubating of the microparticles was aimed at optimizing insulin uptake by the microparticles. The insulin solution was totally imbibed by the microparticles thus the insulin loading efficiency is related to the amount of insulin retained after washing. After incubation in insulin solution, the microparticles were treated with HCl, to effect the closure of the polymer micro-pores by inducing structural collapse of the pH sensitive sodium alginate thus, limiting any possible leaching out of insulin from the matrices of the microparticles. Sodium alginate in aqueous environment forms a tight viscous gel layer that resists swelling and dispersion at low pH values [14]. The gel layer once formed prevents further ingress of water into the polymer matrix. This phenomena result from the reorientation of charges and functional groups within the polymer network that give rise to inter and intra-molecular hydrogen bonding between the oligosaccharides of sodium alginate polymer network and some water molecules. The cellulose acetate coat also contributed in maintaining the insulin within the micro-matrix by limiting the diffusion of fluid in and out of the matrix during the washing process.

Insulin loading generally increased with increasing amount of sodium alginate in the formulation. This is because of the superior entrapment efficiency of sodium alginate relative to mucin when washed with dilute HCl solution. Mucin on the other hand is characterized by a high viscoelasticity than by pH responsiveness. Resistance to loss of insulin from the mucin matrix was mainly due to its high viscosity [16,28]. The higher entrapment efficiency of sodium alginate explains the observed increase in insulin loading efficiency with increase in sodium alginate content of the mucinated sodium alginate microparticles (Fig. 5).

3.5. In vitro release studies

The insulin-loaded microparticles prepared with the different mucin–sodium alginate ratio mixtures were filled into hard gelatin capsules such that each capsule shell contained an amount of insulin equivalent to 100 IU of soluble insulin. Each hard gelatin capsule was placed in a dissolution basket, which was held in place in the dissolution fluid with a clamp and stand. Sink condition was maintained throughout the experiment. The acetate buffer medium was changed from acidic (pH 2.2) to basic (pH 7.4) to simulate the pH environment of the GIT [16,28]. The amounts of insulin released from the capsules were quantified.

The release profile of insulin from the various insulin-loaded microparticles prepared from different mucin–sodium alginate ratio mixtures and filled into gelatin capsules is presented in Fig. 6. The preparations exhibited a lag time before onset of insulin release from the capsulated microparticles. Although there was rapid wetting of the gelatin capsule shell containing the insulin-loaded microparticles in the fluid environment, total disintegration of

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the gelatin capsules shells was generally prolonged. This could be due to the gelation of the mucin–sodium alginate mix, and the subsequent interaction with the gelatin capsule shell that retarded the further permeation of fluid through the capsule shell may contribute to prolong the release of insulin from the microparticles.

The release profiles of insulin from the capsules (Fig. 6) indicate that insulin release from the formulations commenced within 10 min in the dissolution media. This indicates that release of insulin started after fluid penetration of the gelatin capsule shell. The release of insulin in the acetate buffer solution (pH 2.2) despite the coating of the microparticles with cellulose acetate phthalate could be due to the deformation of the cellulose acetate phthalate film coating during the freeze-drying and sieve screening processes. This structural deformation in the microparticles exposed the inner matrix to the dissolution fluid.

The rate of insulin release from the various formulations was evaluated by determining the mean dissolution time (MDT). The MDT of the insulin-loaded microparticles prepared with mucin–sodium alginate ratio mixtures increased with increasing amount of sodium alginate in the microparticulate matrix. The MDT for microparticles prepared with sodium alginate, mucin and mucin–sodium alginate ratios mixtures of 1:1, 3:1 and 1:3 were 11.21 ± 0.75, 3.3 ± 0.42, 6.69 ± 023, 8.52 ± 0.95 and 3.48 ± 0.65 min, respectively. This indicates an increased retardation of insulin release from the microparticles with increase in sodium alginate content relative to mucin. This manner of release is related to the pH responsiveness of sodium alginate [28].

3.6. Blood glucose reducing efficiency

The effectiveness of the oral insulin microparticles prepared with mucin, sodium alginate and the mucinated sodium alginate was assessed based on its effectiveness in blood glucose reduction in diabetic rabbits. The orally administered distilled water and insulin solution as well as the subcutaneously administered insulin solution served as controls. High doses of insulin were administered to the diabetic rabbits in the formulations to allow for possible losses through degradation of the insulin in the GIT [16]. 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.

The percentage reduction of initial glycaemia was used as an evidence of insulin absorption [29] Fig. 7 shows response curves obtained by plotting the percent blood glucose reduction from initial glucose levels versus time. The mean blood glucose baseline (initial glucose level) value was taken as the 100% level and all other blood glucose level/time data were calculated as a percentage of the baseline. In some of the animals the blood glucose levels were higher than the initial levels within the first hour of administration (Fig. 7). This increase could be due to the stress associated with the administration of the capsules [29]. A more efficient reduction in the blood glucose level could have masked this initial increase in other animals. Distilled water generally did not cause any reduction in blood glucose levels. Orally administered insulin solution resulted in a slight fall in the blood glucose level within 2 h of administration, returning to normal at the third hour. The percentage blood glucose reduction for the subcutaneously (sc) administered insulin was significantly ($p < 0.001$) higher than for all the formulations.

Sodium alginate is pH responsive, gelling and swelling comparatively slightly but resisting dissolution in acidic environment [21], while mucin is resistant to proteolytic enzymes and acid pH degradation in the GIT. The insulin-loaded microparticles prepared with the various ratio mixures of mucin and sodium alginate produced blood glucose lowering effect higher than those of either mucin alone or sodium alginate alone. The high blood glucose reduction resulting from insulin-loaded microparticles prepared with the mucinated sodium alginate indicates that there may be synergism between sodium alginate and mucin in insulin protection or absorption within the GIT. The microparticles prepared with one part mucin and three parts sodium alginate produced maximum blood glucose reduction 5 h after oral administration that was equal to that of subcutaneously administered insulin. Fig. 7 also shows a slight reduction in blood glucose level after oral administration of insulin solution. It is quite improbable for insulin absorption to occur in the stomach thus, the reduction in the blood glucose could be due to some of the insulin solution reaching the intestine since high doses of insulin solution were administered to the rabbits. Blood glucose reduction occurred within 1 h 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 mucinated sodium alginate that was efficient in adhering the microparticles to the gastric mucosa and protecting the insulin from degradation [1]. This may also indicate a failure or absence of the enteric coating of the cellulose acetate phthalate. The apparent failure of the enteric coating activity of the cellulose acetate phthalate could have occurred by the structural deformation of

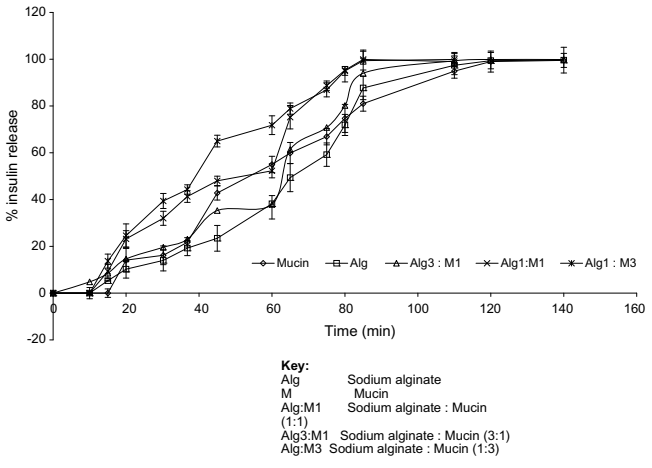

Fig. 6. *In vitro* insulin release from sodium alginate–mucin microparticles.

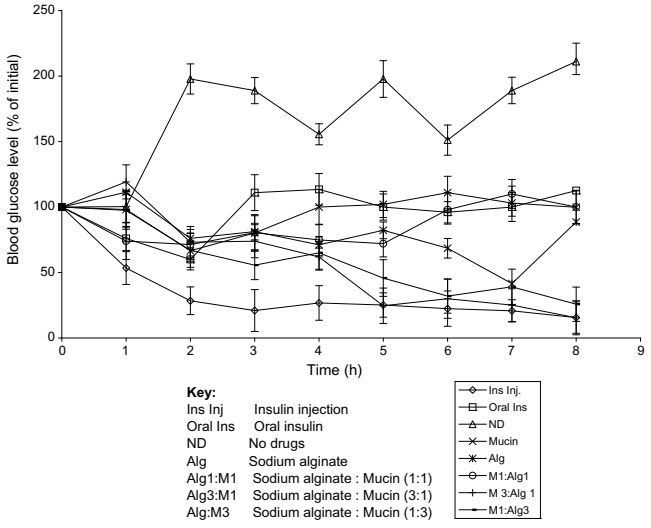

Fig. 7. Percentage reduction in blood glucose level in diabetic rabbits treated with oral insulin loaded in mucin/sodium alginate microparticle formulations.

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the microparticles during the freeze-drying and sieve screening processes.

4. Conclusion

Insulin-loaded microparticles for oral insulin delivery were successfully prepared with sodium alginate, mucin and mucin–sodium alginate ratio mixtures. The results obtained from our studies show the effectiveness of the mucinated sodium alginate as a carrier system for oral insulin delivery. The samples prepared with three parts of sodium alginate and one part of mucin produced blood glucose lowering effect comparable to that of the subcutaneously administered insulin after five hours. This indicates that oral delivery of insulin for effective control of blood glucose is indeed possible using the right carrier system and formulation technique.

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