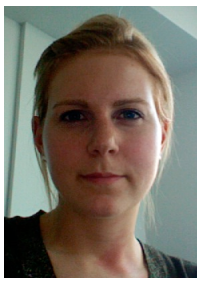
Alginate microparticles produced by spray-drying for oral insulin delivery



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Introduction

In spite of the prevalence of subcutaneous injections for insulin administration, there are several concerns associated with this mode of administration including injection anxiety, pain, cost, infection, and as a result an overall decrease in patient compliance (Khafagy 2007). This has prompted researchers to search for alternative delivery methods including transdermal, nasal, pulmonary, rectal and oral administration routes (Damge 2007; Sadrzadeh 2007). Peroral delivery is particularly interesting because it offers many advantages including ease of administration and high patient compliance (Chalasani 2007). Good patient compliance and tight glycemic control is correlated with a decrease in complications such as blindness, lower limb amputations and kidney failure. This could potentially improve the quality of life for millions of diabetics. Additionally, oral delivery offers the unique feature of being able to mimic the physiological path that insulin would travel by entering the hepatic portal vein from the intestine and then to the liver (Raj 2003). In contrast, insulin injected subcutaneously must circulate through the body before reaching the liver. Accordingly, insulin delivered directly to the liver could decrease complications, such as atherosclerosis, which are associated with high concentrations and build-up of insulin in the body.

Despite the clear advantages of oral insulin, currently, there is no commercially available delivery system on the market today due mainly to its low bioavailability in the gastrointestinal tract (GIT) (Sadrzadeh 2007). There are two principal factors that are attributed to this low bioavailability: enzymatic degradation and poor absorption across the epithelial lining of the GIT (Hamman 2005). Polymeric encapsulation offers a promising method to overcome these obstacles by protecting insulin in the GIT as well as increasing permeability into systemic circulation. Spray drying, the technique employed in this study, is an advantageous encapsulation technique compared to other nanoparticle formulation techniques, such as ionotropic pre-gelation and nanoemulsion dispersion, because particles can be produced relatively quickly and the solution can be fed continuously. It can take over a day to produce particles from nanoemulsion dispersion, whereas they can be formulated in a couple of hours using the spray-drying technique. Additionally, spray-drying is a process that is already commonly used in the food and pharmaceutical industries.

Alginate is made up of alternating patterns of 1,4'-linked β-D-mannuronic acid (M) and α-L-guluronic acid residues (G). It was selected as the core polymer because it is natural, biocompatible and biodegradable. It is an ideal encapsulation matrix for nanoparticles due to its availability and particular chemical and physical properties. It is soluble in water, the encapsulation process is free of organic solvents, and the porosity can be controlled with simple coating procedures.

The purpose of this study was to produce alginate nano and microparticles via spray-drying and assay them for characteristics such as size, morphology, protein loading, and insulin release.

Materials and Methods

Materials

Novolin GE Toronto, a human recombinant insulin from Novo Nordisk (100 IU/mL), was purchased from a local pharmacy (Kingston, Ontario, Canada). Low viscosity sodium alginate (viscosity of 2% solution at 25°C, 250 cps) was purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). All other reagents were of chemical grade.

Methods

Particulate Formulation

Insulin (100 IU/mL, 10 mL) was added to a 1, 1.5 or 2% w/v alginate solution and gently mixed for 5 minutes with a magnetic stir bar at 250 rpm. The dispersion was then fed into a Büchi Mini Spray Dryer B-290 at a feed rate of 5 mL/min, an atomization air flow rate of 600 L/hour, an inlet temperature of 150°C and an aspirator rate of 38 m^3/hour. The resulting dry particles were collected and stored at 2-6°C. Polymeric particles were also prepared without insulin to use as controls.

Size Distribution

The size distribution was determined by laser diffraction spectrometry using a *Malvern Mastersizer 2000* with the dry particle accessory, *Scirocco 2000.*

Morphology

Samples of the particles produced were mounted, coated with a thin layer of gold and analyzed using a scanning electron microscope (JOEL, JSM-840). Several micrographs were obtained in order to determine the shape, surface profile and rough size estimates of the particles.

Encapsulation Efficiency

The encapsulation efficiency (EE) was determined by suspending 150 mg of particles in a solution of phosphate buffer (pH=7.4). The suspension was incubated at room temperature under magnetic stirring (250 rpm) to dissolve the particles. After 2 h, a sample of the solution was removed and centrifuged (2000*g*, 10 min). The protein content was quantified by UV absorbance (280nm). The expression for EE is shown in equation 1.

$$EE = \frac{Insulin\ mass\ released}{Initial\ mass\ of\ insulin\ used} \times 100 \qquad (1)$$

In vitro Gastrointestinal Simulations

In order to simulate insulin release from the particles in the gastrointestinal tract, 10mg of particles with an initial alginate concentration of 2% were put into 0.5 mL of a hydrochloric acid solution (pH=1.2) or 0.5mL of a phosphate buffer at an intestinal of pH 6.8. The suspensions were placed into an incubator at 37°C and stirred by an orbital shaker at 100 rpm. At appropriate time intervals, aliquots were removed and centrifuged (12500g, 10 min). The insulin concentrations were assayed using UV absorbance (280nm).

Results and Discussion

Particles were sized and the mean size, regardless of initial polymer concentration, was determined to be 1.83±0.08μm. This mean size is acceptable for oral insulin delivery because it has been shown that particles several microns in diameter (but less than 10 μm), are absorbed by the M cells of the Peyer's patches in the intestine (Chen 1998). The spray dryer operates by capturing the particles in a cyclone type collector, but it is apparent, that because of the small size of the particles, a

significant fraction are being lost in the effluent air stream. Improvement options such as electrostatic collectors and feed additives are currently being evaluated to improve mass yield.

Particle morphology, as seen in figure 1, was determined by coating particles with a gold film and imaging with a scanning electron microscope.

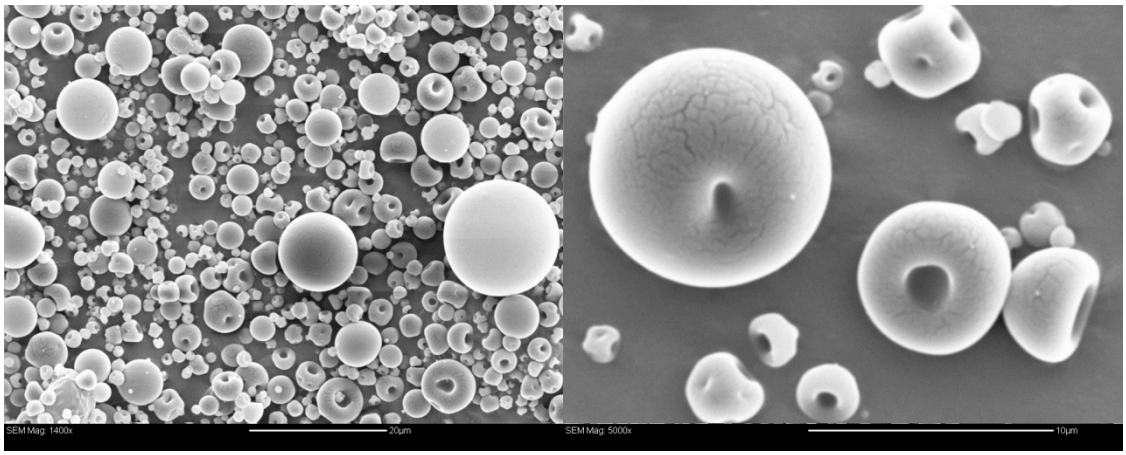

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Figure 1 – Micrographs of spray-dried alginate-insulin particles.

It can be seen in figure 1 that the particles are spherical with dimpled surfaces. Upon further research, it appears that the divots are an artifact of the spray-drying process and not a major concern; however, through various formulation techniques, it is possible avoid these divots.

The encapsulation efficiency or particle loading was determined by dissolving the particles in neutral buffer, and assaying supernatant protein at 280 nm as shown in table 1. Encapsulation efficiencies ranged from 16 to 26% depending on initial alginate concentration, but are generally low. EE is presently being confirmed using ELISA immunoassay.

Alginate Formulation	Encapsulation Efficiency (%)
1%	16.5 ± 2
1.5%	25.9 ± 2
2%	26.3 ± 4

Table 1 – Encapsulation efficiencies assayed by UV absorption (280nm)

The *in vitro* gastrointestinal simulation was conducted in order to determine how the particles behave in gastric and intestinal environments. Figure 2 shows the cumulative insulin release in gastric and intestinal pH for particles produced from a 2% initial alginate concentration.

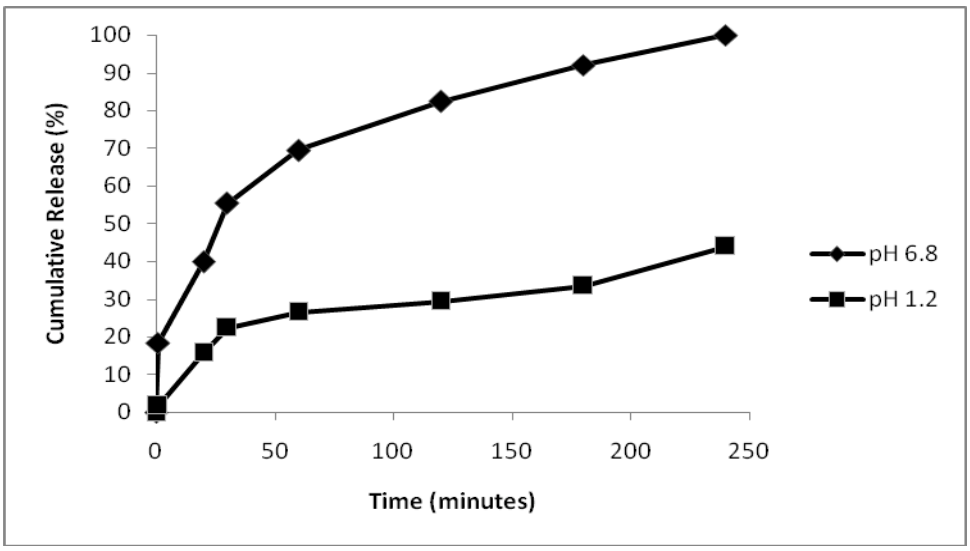

Figure 2 – Cumulative insulin release in alginate microparticles produced by spray-drying. Protein concentrations were assayed by UV absorption of supernatant (280nm).

Figure 2 shows that approximately 40% of the insulin is released in the acidic environment, and fully released in the basic "intestinal" simulation. The next step in this assay will be to simulate particle transition and residence time in the gastrointestinal tract. Particles will be initially suspended in gastric fluid for 2 hours and then resuspended in intestinal fluid for another 4 hours.

Conclusions

This study shows that it is possible to formulate alginate microparticles using a lab-scale spray-dryer for oral insulin delivery. The particles were produced and assayed for size, morphology, encapsulation efficiency and protein release in gastric and intestinal mediums. The size and morphology are consistent with the characteristics necessary for oral insulin delivery; however future work must be done to increase the encapsulation efficiency of the particles by altering the core formulation or through the addition of polymer coats. Additionally, the protein release experiment will be improved in order to further determine how particles will behave in the gastrointestinal tract.

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