Delivery of Encapsulated Human Recombinant Crystalline Insulin From PLGA Microspheres

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INTRODUCTION

Since the advent of insulin, biopharmaceuticals have become an increasingly popular therapeutic intervention against a variety of medical conditions including obesity, cancer and diabetes (Stevenson C.L., 2009). According to Walsh (2005), biopharmaceuticals are classified as recombinant therapeutic proteins, monoclonal antibodies or nucleic acids. Currently, up to 151 recombinant biopharmaceuticals have been approved by the Food and Drug Administration (FDA) and the list of submissions continues to grow (Ferrer-Miralles N., 2009).

While there is rapid growth in the field of biopharmaceutical research, one major pitfall continues to challenge this area of drug development. Currently, most biopharmaceuticals are delivered via intravenous infusion, or subcutaneous injection, as is the case with insulin (Degim I.T., 2007). Unfortunately, these dosage forms often cannot protect the peptide from enzymatic degradation and therefore require frequent dosing to overcome this issue.

Poly(lactide-co-glycolide) microspheres on the other hand, are one example of a dosage form which can provide sustained peptide release for several weeks (Taluja A. et al., 2007). Currently, PLGA is used in several FDA medical devices including Lupron Depot® which has demonstrated superior efficacy and biocompatibility (Mittal G. et al., 2007). One downside to this technology, however, is that the encapsulation process is often too harsh for many peptides (Sanchez A. et al., 1999). Alternatively, Morita T. et al. (2000) have reported that encapsulating crystallized peptides using a solid-in-oil-in-water (s/o/w) technique may overcome this potential problem.

The objective of this study is therefore, to examine the potential of using an s/o/w encapsulation method to encapsulate human recombinant crystalline insulin in PLGA microparticles. Various insulin loadings will be tested to determine the encapsulation efficiency, particle and pore size distribution, insulin release kinetics and morphology. Lastly, DSC analysis will shed light on the effect of insulin loading on polymer interactions. These data, will therefore determine whether this technique is a viable encapsulation approach for future biopharmaceuticals applications.

MATERIALS and METHODS

PLGA (*L*,*G* 50:50, 5-15kDa), crystalline human recombinant insulin, poly(vinyl alcohol) (PVA), LR white embedding kit and sodium phosphate dibasic anhydrous (Sigma Aldrich, Oakville). BSA and Micro BCA Protein Assay Kit (ThermoFisher, Ottawa). Dichloromethane (DCM) (Caledon Labs, Georgetown).

Preparation of PLGA Microparticles – Four different 100 mg batches of PLGA microparticles were prepared with 0%, 2.5%, 5% and 10% w/w crystalline insulin. PLGA was dissolved into 1 mL of DCM and added to a vial containing crystalline insulin to form the primary s/o suspension. The suspension was the added drop-wise to a separate beaker containing 30 mL 10% PVA solution pre-

chilled to approximately 5-10°C. An impeller mixer (Caframo, Wiarton), stirred the resulting s/o/w emulsion under constant mixing at 430 rpm for 1 min. After mixing, the emulsion was transferred into a beaker filled with 500 mL pre-chilled distilled water set stirring at 180 rpm for 10 hrs on ice. The microparticles were then isolated by vacuum filtration, dried in the fridge for 12 hrs and vacuum dried for 5 hrs. All microparticles were later stored in the freezer.

Encapsulation Efficiency (E.E.%) – E.E.% was measured using a method described by Meinel et al. (2001). Briefly, 3 mL DCM was added to 8-10 mg of the microparticles. After the DCM had evaporated, 20 mL of 5% SDS, 0.1 M NaOH solution was added (VWR, Mississauga). Samples were then stirred for 24 hrs at 37°C and 80 rpm with a magnetic stirrer. Supernatants were removed and analyzed spectrophotometrically using a micro BCA protein assay kit along with BSA standards. E.E. % was defined as a quantity of insulin measured by the assay versus the quantity of insulin added during manufacturing. All samples were measured in triplicate.

Sizing and Morphology Analysis– Particle and pore size distributions were determined by hand using images captured by SEM (JEOL 840, USA). TEM (JEOL 1200EX, USA) imaging of the internal morphology was determined by microtoming microparticles embedded within LR white resin.

Insulin In Vitro Release Study – Using a method adapted from Meinel L. et al. (2001), 5 mg of microparticles were placed into 2.5 mL plastic centrifuge tubes (Diamed, Mississauga) and filled with 2 mL 20 mM phosphate buffer, pH 7.4. All samples were incubated at 37°C on a flat orbital shaker rotating at 60 rpm. The supernatants were collected at various time points, measured using a micro BCA protein assay kit and compared to BSA standards. Release experiments were performed in triplicates.

DSC Analysis – 4-6 mg of microparticles were placed into aluminum pans which were hermetically sealed. Both empty and sample pans were placed into a DSC (TA Instruments, USA), and cooled to -30°C and equilibrated for 1 min. Samples were then heated to 110°C and cooled back to -30°C at rate of 10°C per min. This process was repeated three times.

RESULTS and DISCUSSION

E.E.% was measured to determine optimal loading concentrations for PLGA microparticles. Table 1 indicates that the highest encapsulation efficiencies were achieved when insulin loading was low. Conversely, lower insulin loadings exhibited the highest degree of E.E% variance. This variation emanates from the imprecision encountered during the weighing of raw materials.

Sizing analysis (Table 1) performed on the microparticles and their pores reveals that the mean diameters remained relatively consistent as insulin loading increased. Crystalline insulin was also noted to be approximately 2.5% the mean diameter of the microparticles across all batches.

Formulation	0% Insulin	2.5% Insulin	5% Insulin	10% Insulin	Crystalline Insulin
E.E.%	0	99% ± 10%	83% ±8%	78% ±1	-
Mean Diameter µm	154 ± 57	131 ± 92	153 ± 53	137 ± 52	4 ± 5
Mean Pore Size nm	318 ± 121	282 ± 106	314 ± 126	387 ± 145	-

Table 1: E.E.% and sizing analysis for various batches of PLGA microparticles.

Scanning and transmission electron microscopy reveal smooth surfaces with small porous structures appearing on the surface and within the outermost crust of the microparticles. Figure 1 illustrates microparticles with insulin loadings ranging from 0-10% respectively.

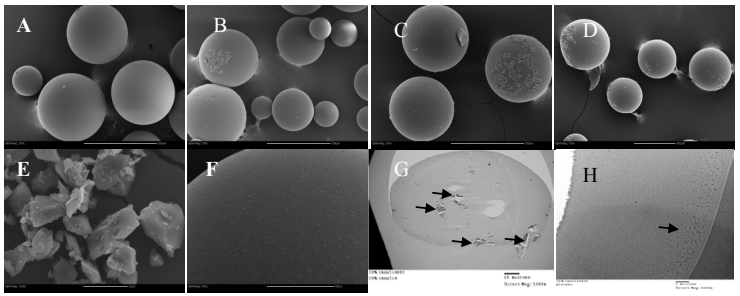

Figure 1: A) 0% insulin microparticles, B) 2.5% insulin microparticles, C) 5% insulin microparticles, D) 10% insulin microparticles, E) insulin crystals, F) porous outer surface of 0% insulin microparticles, G) TEM cross section of a 10% insulin microparticle (arrow indicates insulin crystals and H) cross section of outer crust of 0% insulin microparticles (arrow indicates porous region).

Figure 2. illustrates the release profiles for microparticle formulations containing 2.5, 5 and 10% w/w insulin loadings. Similar biphasic release kinetics are attributed to the bulk erosion process of PLGA matrices (Mittal G. et al., 2007).

DSC analysis was performed to determine the influence of insulin loading on T_g of the PLGA microparticles. Figure 3 illustrates that as the insulin loading increased, the T_g of the PLGA also increased.

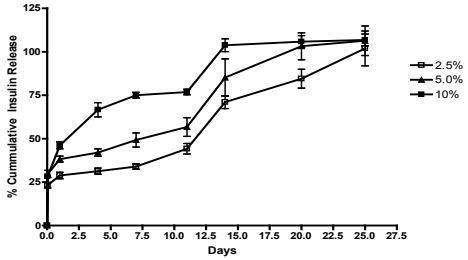

Figure 2: Cummulative insulin release profiles for microparticles loaded with 2.5, 5 and 10% crystalline insulin.

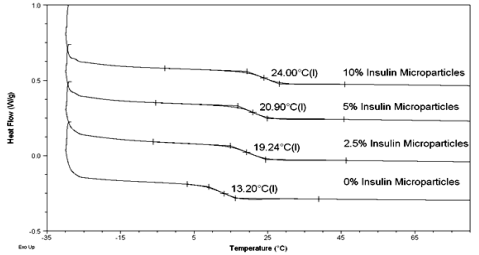

Figure 3: DSC thermograms for blank and loaded microparticles

CONCLUSIONS

These results indicate that low loadings of crystalline insulin can be encapsulated within PLGA microparticles with high encapsulation efficiencies and provide sustained release for up to 25 days. Analysis of the bioactivity of released insulin is presently ongoing.

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