Zinc Phosphate-Insulin-Alginate Particles for Oral Insulin Delivery: Feasibility Studies

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Statement of Purpose: Type I diabetes is characterized by the inefficiency of pancreatic beta cells to produce insulin. The common form of insulin therapy is by way of twice daily subcutaneous insulin injection. Various attempts have been made to develop a non invasive delivery system for insulin namely, via oral, buccal, transdermal delivery routes etc with varying levels of success. It is known that divalent metal cations form complexes with helical phosphates of DNA, which stabilises the structure. Similarly, the two protein subunits in each insulin molecule will be bound to a single zinc atom with a significant increase in insulin stability. Therefore zinc phosphate nanoparticles could be utilized for attaching insulin and deliver via oral route with suitable pH sensitive sustained release coating which can protect insulin particles from hydrolysis and enzymatic degradation. We have attempted to develop zinc phosphate particles with average size of 200nm, attach insulin to these particles and coat with pH sensitive sodium alginate coating.

Methods: Zinc phosphate nanoparticles were prepared by precipitation of zinc in a phosphate solution (2). These particles were ultrasonicated for about 15 min at 100W. The particles obtained were washed with distilled water and freeze dried. The particle size distribution was evaluated using zetasizer nanoseries (Malvern Instruments). (Insulin (Human insulin 400 IU/ml) was added to the dry particle and kept overnight for insulin binding at 4°C. Insulin bound zinc phosphate nanoparticles were centrifuged to remove excess insulin and dried in a refrigerator. These particles were dispersed in a 1%, 2% and 4% solution of sodium alginate, and dried under refrigerated condition after centrifugation to obtain particles with three different coating. In vitro release kinetics were evaluated in simulated gastric (SGF, pH 1.2) and intestinal (SIF, pH 6.8 and 7.4) fluids at 30 °C. Insulin activity was evaluated by ELISA technique.

Results / Discussion: Particle size distribution of zinc phosphate nanoparticles is given in figure 1. Particle size ranges from 190 to 295nm with a peak diameter of 226nm.

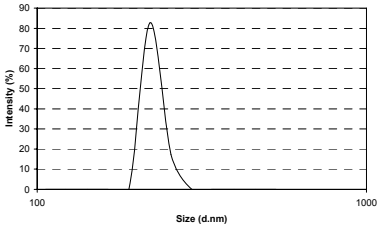

Figure 1: Particle size distribution of zinc phosphate nanoparticles

Insulin loaded particles were dispersed in 1%, 2% and 4% solutions of sodium alginate for alginate coating. The size of these coated particles was much bigger and was possibly in the range of 1 micron as observed through an optical microscope. Insulin loading in the 3 formulations was evaluated by extracting the loaded insulin into phosphate buffer pH 7.4 and estimating the protein content by Lowry's method. The activity of the extracted insulin was evaluated by ELISA technique which was observed as 100%. The drug loading was 23 IU/100mg, 22 IU/100mg and 21 IU/100mg respectively for 1%, 2% and 4% chitosan coating. The release of insulin in vitro into simulated gastric as well as intestinal medium is shown in figure 2.

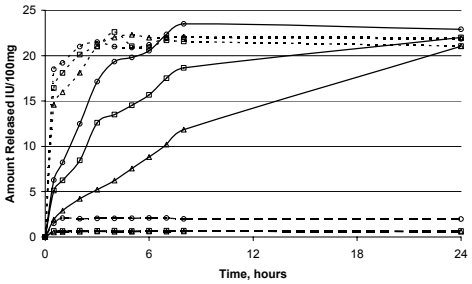

Figure 2: Amount of insulin released into SGF (pH 1.2, ––––), SIF (pH 6.8, ——) and SIF (pH 7.4, ------); 1% - circle, 2% - square and 4% triangle.

In the gastric medium the release of insulin was negligible for all coatings and in pH 7.4 the release was almost instantaneous with 100% insulin release in the first 3 hours itself. However, in pH 6.8 (SIF as per USP) the release of insulin was sustained with significant sustenance for 4% coating. The uptake of particulate carriers by the gastrointestinal epithelium has encouraged number of research groups to investigate nanoparticulate delivery for insulin (3). Translocation of alginate coated zinc phosphate particles through the intestinal lymphatic system may be used as a system for de;ivering insulin via oral route. In vivo studies need to be completed to evaluate the uptake of particles through the intestinal epithelium.

Conclusions: We were able to prepare pH sensitive ceramic insulin particles from zinc phosphate nanoparticles and demonstrate its release profile in vitro. The result was promising towards its possibility in the development of a non-invasive oral insulin delivery system for diabetes. However, the question of degradability of the ceramic particles is yet to be studied.

References:

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