Amphiphlic Zinc Calcium Phosphate Nanoparticles for Oral Insulin Delivery

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Statement of Purpose: Injections had been the only available route for delivery of insulin since its discovery by Banting and Best in 1921. Oral delivery of insulin can replace daily injections to diabetic patients, however, poses unique problem of stability and susceptibility to proteolysis, which reduce their bioavailability. Various investigations to improve oral bioavailability of insulin by delivering via ceramic and polymeric nanoparticles are ongoing (1). Nanoparticles are utilized for drug delivery applications because of its, high stability, prolonged residence time, high drug encapsulation, better storage life and translocation of nanoparticles through the intestinal barrier;by paracellular pathway or via M cells in Peyer's patches (2). Many crystalline modifications of insulin have been identified but only those with the hexamer as the basic unit are utilized in preparations for therapy as the insulin hexamer forms a relatively stable unit in the presence of zinc ions. An attempt has been made to develop self-aggregated nanoparticles of lauric acid and polyethylene glycol incorporating zinc phosphates. Amphiphilic nature and the presence of zinc phosphate help efficient incorporation of insulin without denaturization and helps efficient translocation across the intestinal epithelium.

Methods: Zinc calcium phosphate nanoparticles were prepared by co-precipitation of zinc chloride and calcium chloride in a phosphate solution (3). Simultaneously lauric acid and O,O'-Bis(2-aminopropyl) polyethylene glycol 1900) was dissolved in dimethyl formamide in the presence of N,N'-dicyclohexylcarbodiimide. After 24 hours of incubation, this is precipitated in water and dried in an oven. This was redissolved in DMF along with the zinc phosphate nanoparticles to form a uniform suspension. Self-aggregates were prepared by addition of this suspension into insulin solution (400IU/ml obtained from USV Ltd. Mumbai India). The particle size distribution was evaluated using zetasizer nanoseries (Malvern Instruments). These nanoparticles were encapsulated in calcium alginate to impart pH sensitivity. The insulin loading and the release profile was evaluated in simulated gastric (SGF, pH 1.2), and intestinal fluids (SIF, pH 6.8). The released insulin was analysed for stability utilizing Nano ZS.

Results/Discussion: Particle size distribution of zinc phosphate nanoparticles and self-aggregated nanoparticles are given in figure 1. Particle size was 190nm for zinc phosphate nanoparticles and 224nm for self-aggregated nanoparticles with insulin. Insulin loading of self aggregated nanoparticles was 23 IU/10mg. The release of insulin in vitro into simulated gastric as well as intestinal medium from alginate encapsulated self-aggregates is shown in figure 2.

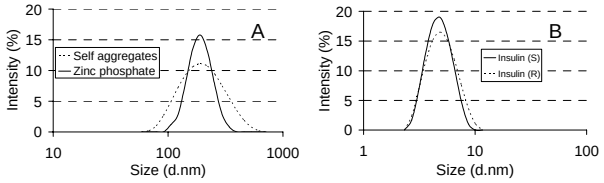

Figure 1: a) Particle size distribution of zinc calcium phosphate and self-aggregated nanoparticles. b) Hydrodynamic diameter of insulin solution and released insulin from the nanoparticles.

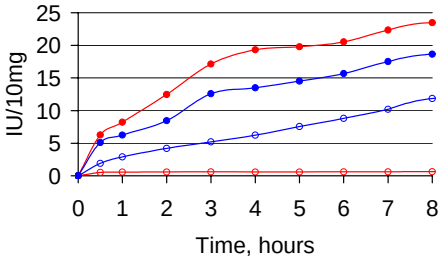

Figure 2: Amount of insulin released into SGF (red), SIF (blue) from Self-aggregated nanoparticles; alginate coated (open circle); uncoated (solid circle).

Alginate coating was done because self-aggregated particles did not show any pH sensitiveness. For alginate-coated particles, the release of insulin in gastric fluid was negligible. However, in simulated fluid release was sustained for over 8 hours. Although uncoated self-aggregates also exhibited sustained release pH sensitiveness was required to by pass the hostile conditions of the stomach. The hydrodynamic size of insulin solution before loading (Insulin-S) and the released insulin (Insulin-R) from the self-aggregates are similar and is 5.37nm (figure 1b). It shows that the insulin is in a hexameric form and the released insulin is stable with no visible aggregation. Translocation of amphiphilic self aggregates of lauric acid and PEG incorporating zinc calcium phosphate through the intestinal lymphatic system may be used as a system for delivering insulin via oral route, since fatty acid incorporation helps in effective uptake of particles through the intestinal epithelium.

Conclusions: It has already been established that fatty acid complexation can improve uptake of particles across epithelium (2). Present study demonstrates that self-aggregates of lauric acid and peg incorporating zinc calcium phosphate nanoparticles can be used as a carrier for delivering insulin orally. Conformational changes of released insulin from the particles were insignificant compared to the native insulin.

References:

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