Self Assembled Nanoparticles based on Chitosan and Anacardic acid 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 insulin via chitosan nanoparticles are ongoing along with other bioadhesive polymers (1). The positive charge of chitosan nanoparticles and its bioadhesiveness has been capitalized for the increased bioavailability of insulin. 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). Anacardic acid, a natural fatty acid with long aliphatic side chain, is a cell permeable salicylic acid analog that displays a variety of biological activities, such as antimicrobial. It is a natural aspirin derivative which has shown bioactivity (3). An attempt has been made to develop self-aggregates of hydrophobically modified chitosan using anacardic acid and to utilize these nanoparticles for delivering insulin orally.

Methods: Anacardic acid was coupled with chitosan using thionyl chloride reaction (4). This was filtered, precipitated in ethanol and dried. The product was characterized using FTIR. N-anacarddoylated chitosan was dissolved in DMF and added to the insulin solution (400IU/ml obtained from USV Ltd. Mumbai India) to obtain insulin-loaded chitosan self aggregates. The particle size and zeta potential of these self-aggregated nanoparticles were analyzed using MALVERN Nano ZS. The insulin loading and the release profile was evaluated in simulated gastric (SGF, pH 1.2), and intestinal fluids (SIF, pH 6.8). The protein content was estimated using Lowry's method. The released insulin was analysed for stability and conformational variations with Nano ZS and Circular Dichroism (Jasco J-810 spectropolarimeter).

Results/Discussion: The FTIR spectrum of the N-anacardoylated chitosan showed strong absorptions at 1772 cm^{-1} and 1646cm^{-1} indicating N-amidation. As shown in figure 1(a), the Z-average particle size of the self-aggregated nanoparticles was 214 [d.nm]. The nanoparticles were having a net negative charge with a mean zeta potential of -20.3 mV at neutral pH. The total insulin load in these nanoparticles was 10±0.5 IU/mg of dried nanoparticles. Since these modified chitosan nanoparticles swells in acidic pH, complete insulin was released in SGF in 15 minutes (figure 2). Therefore these insulin loaded modified chitosan nanoparticles were encapsulated in calcium alginate to impart pH sensitiveness. Alginate coated nanoparticles released no insulin in the gastric fluid; however, in intestinal fluid it demonstrated a sustained release of over 8 hours.

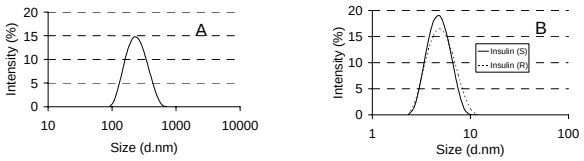

Figure 1: a) particle size of chitosan-anacardic acid self aggregates, b) hydrodynamic diameter of insulin solution (line) and released insulin from the nanoparticles (doted)

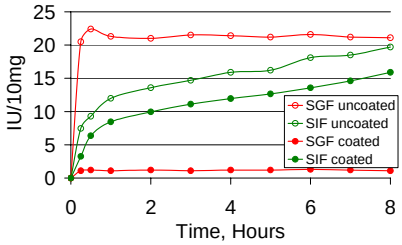

Figure 2: Amount of insulin released from chitosan nanoparticles in simulated gastric (SGF) and intestinal (SIF) fluids

The hydrodynamic size of insulin solution before loading (Insulin-S) and the released insulin (Insulin-R) from the chitosan nanoparticles 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. CD deconvolution and predicted secondary structure (SELCON method) of Insulin-S and Insulin-R indicates no conformational changes on incorporating into chitosan nanoparticles (table 1).

Table 1: CD Deconvolution and predicted secondary structure

Sample	Fractional composition (%)			
	α-helix	β-strand	Turns	Random
Insulin-S	35	12	23	31
Insulin-R	34	12	23	31

Conclusions: It has been demonstrated that self aggregated nanoparticles from hydrophobically modified chitosan using anacardic acid can be utilized as oral insulin delivery system. The insulin released from the particles was stable with no conformational changes. It has already been established that fatty acid complexation can improve uptake of particles across epithelium (2). Since anacardic acid is a natural fatty acid and an aspirin derivative, these nanoparticles may be translocated efficiently across the intestinal epithelium and may also be biocompatible.

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