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Short communication

Synthesis and characterization of alginate coated zinc calcium phosphate nanoparticles for intestinal delivery of insulin

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ABSTRACT

Nanosized calcium phosphates studied as drug delivery systems are highly compatible with the various drugs like insulin, antibiotics etc. Zinc is an essential trace element that plays a crucial role in the synthesis, storage and release of insulin in a human body. Therefore, an attempt has been made to develop zinc modified calcium phosphate nanoparticles (less than 100 nm) as carriers for intestinal delivery of insulin. The insulin loaded nanoparticles were coated with pH sensitive alginate. These pH sensitive nanoparticles released insulin in the intestinal medium, and the conformation of released insulin was stable. The blood glucose level of diabetic rats came to normal on administration of the formulation. With the beneficial effect of zinc reported on diabetic patients, the present system seems to be an excellent carrier for intestinal delivery of insulin.

1. Introduction

Nanostructured materials pose unique properties and capabilities that make it suitable, for interaction with the biological activity particularly in drug delivery applications [1]. Low chemical stability, drug release rate that is inappropriate to the application, risk of microbial contamination, and the undesirable effects of the organic solvents used for particle development, are some of the inherent problems of organic nanoparticles [2–4]. Inorganic nanoparticles are non-toxic, hydrophilic, biocompatible and highly stable compared to organic materials [5]. Inorganic nanoparticles as drug or gene delivery carriers have been received much attention due to their high cellular uptake capacity, non immunogenic response, and low toxicity [6]. Synthetic calcium phosphates possess excellent biocompatibility and bioactivity properties with respect to bone cells and tissues, therefore, have been widely used clinically in the form of powders, granules, dense and porous blocks and various composites [7]. Nanosized calcium phosphates have been studied in drug delivery systems like intestinal delivery of insulin [8,9], or other drugs such as antibiotics [10].

Ceramics as potential carriers for protein or polypeptide drugs is being widely explored recently because of its many beneficial properties over polymeric drug carriers [7]. Calcium phosphate has been proved to be highly compatible with insulin [11]. Calcium phosphates have also been approved for human use in several European countries as adjuvant. Zinc is being used for stabilizing insulin (long acting insulins) [12]. Since zinc plays a prominent role in the synthesis, storage and secretion of insulin as well as conformational stability of insulin in the hexameric form [13], zinc modified calcium phosphates seem to be a suitable candidate for developing ceramic based insulin delivery systems. Zinc also has insulin-like effects on cells, including promotion of both lipogenesis and glucose transport [14]. The objective of the present work was to develop zinc modified calcium phosphate nanoparticles which could be efficiently used as carriers for intestinal delivery of insulin. The nanoparticles developed were loaded with insulin and coated with a pH sensitive alginate. These nanoparticles were characterized for the application towards its utilization as a carrier of insulin. In vitro release kinetics from these coated nanoparticles was studied. The conformational stability of the released insulin was also evaluated. Lowering of blood glucose level in induced diabetic rats on administration of this formulation was studied. From the present study, it seems that zinc modified calcium phosphate nanoparticles are excellent carrier system for the intestinal delivery of insulin.

2. Materials and methods

Human insulin (400 IU/ml) was a generous gift from USV, Mumbai, India. Sodium alginate (2% solution at 25 °C, 250 cps) was from Sigma Chemical Co. St. Louis, USA. All other chemicals used were of analytical reagent grade. Zinc calcium phosphate (ZnCaP) nanoparticles were prepared by a similar approach as reported [15] with slight modification (supplementary data).

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2.1. Characterizations of nanoparticles

Nanoparticles were characterized by estimating its particle size and zeta potential (Zetasizer Nano ZS and MPT-2 autotitartor, Malvern Instruments Limited, UK); Fourier Transform Infra Red (FT-IR) Spectroscopy (Nicolet Impact 410) and X-ray diffraction (XRD). The in vitro cytotoxicity of the nanoparticles was evaluated by MTT assay [16] done on mouse fibroblast (L929) cell lines as per the directions of ISO standard [17]. Raman spectra and Raman spectral chemical mapping of alginate coated nanoparticles were recorded using Witec alpha-300R confocal Raman microscope equipped with a laser emitting at 532 nm with a laser power of 40 mW (supplementary data).

2.2. Insulin loading, alginate coating and in vitro release kinetics

Insulin was loaded into CaP and ZnCaP nanoparticles by diffusion filling process and coated with <0.5 ml of 1%, 2% and 4% aqueous sodium alginate solutions to develop ZnCaP-1, ZnCaP-2 and ZnCaP-4 respectively. Insulin loading capacity and release kinetics of insulin loaded alginate coated nanoparticles in Simulated Gastric Fluid (SGF pH 1.2), Simulated Intestinal Fluid (SIF pH 6.8) and PBS (pH 7.4) were evaluated (supplementary data) [18].

2.3. Radioimmunoassay, circular dichroism and dynamic light scattering studies on released insulin

Samples of released insulin from CaP, ZnCaP and ZnCaP-4, were subjected to RIA to determine its immuno-reactivity. RIA was performed following the Coat-A-Count Protocol. The radioactivity was measured by counting the tubes in a gamma counter (1470 Automatic Gamma Counter, Perkin Elmer Wizard). Conformational stability of the released insulin was studied using Circular Dichroism (Jasco J-810 spectropolarimeter) using a 1-cm path-length quartz cell at a protein concentration of 2 mg/ml (supplementary data).

2.4. Administration of formulation to diabetic rats

Prior approval of the protocol has been taken from the institutional animal ethics committee, and the experiments were conducted according to the current, relevant national guidelines. Fasting adult Wistar rats having an average weight of 230 g were induced with diabetes by giving a single intra-peritoneal injection of streptozotocin (60 mg/Kg). Diabetes was confirmed by evaluating the blood glucose level (BGL) on the fourth day. The average weight and BGL of diabetic rats were about 200 g and 325 mg% respectively. Before the experiment was initiated, the diabetic rats were fasted for 16 h. Animals were administered with an amount of ZnCaP-4, which is equivalent to 6 IU of human insulin utilizing a feeding tube which is directly emptying into the stomach. Free access to water was allowed to all animals. BGL level of animals was estimated using enzymatic method before oral administration and at specified time intervals.

3. Results

3.1. Particle size and cytotoxicity

The zinc calcium phosphate nanoparticles prepared by the current procedure had a D50 of 86.3 nm (D90 = 112 nm) with a poly dispersity index of 0.051. The particle size varied between 40 and 200 nm with a significantly larger number of particles falling below 100 nm (Fig. 1). The D50 of CaP nanoparticles were 47.9 nm, however, had a large particle size distribution (D90 = 152 nm) and poly dispersity index of 0.165. The insulin loaded ZnCaP nanoparticles were coated with sodium alginate. The free calcium ions present in the particles helped to cross link sodium alginate to form calcium alginate. These nanoparticles were non cytotoxic as shown in Fig. S1 (supplementary data).

3.2. Characterization by FTIR and XRD

The FTIR spectrum of the zinc calcium phosphate (ZnCaP) nanoparticles (Fig. S2) shows the characteristic peaks of PO_4 bend at 582 cm^{-1} and 601 cm^{-1}, CO_3^- group at 872 cm^{-1}, PO_4 stretch at 961 cm^{-1}, PO_4 bend at 1035 cm^{-1} and adsorbed H_2O at 3444 cm^{-1}. The nanoparticles prepared were crystalline and confirmed to be zinc calcium phosphate from the standard XRD data (JCPDS File #9-432 and JCPDF33-1474) as shown in Fig. S3 [19]. The peaks at 24.4° (2 0 1), 25.6° (2 4 0), 27.6° (1 4 1), 29.2° (0 6 0), 29.6° (2 5 0), 30.1° (0 5 1), 34.3° (3 3 1), 35.7° (0 0 2), 36.7° (1 0 2) and 38.4° (2 6 1) corresponds to zinc phosphate hydrate. The peaks 21.8° (2 0 0), 22.9° (1 1 1), 31.8° (2 1 1) and 39.3° (2 1 2) corresponds to calcium phosphate hydroxide.

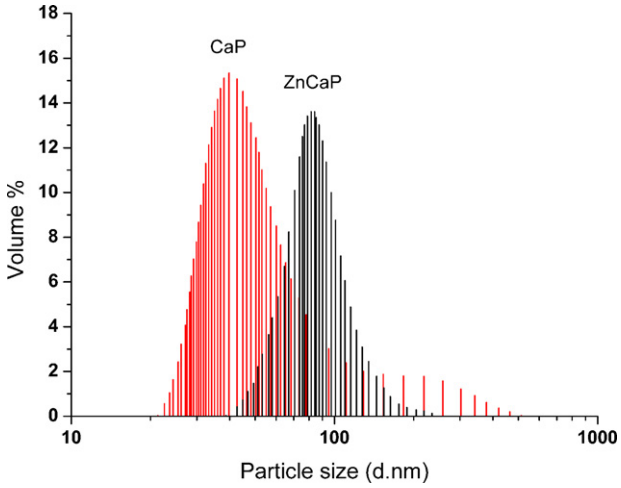

Fig. 1. Particle size histogram of calcium phosphate (CaP) and zinc calcium phosphate (ZnCaP) nanoparticles determined by dynamic light scattering.

3.3. Zeta potential titration

In an oral delivery system, the formulation or carrier would be subjected to a diverse physiological pH conditions as it passes from the stomach to the intestine. Therefore, the changes in zeta potential of the alginate coated particles were studied at different pH conditions by titration. The zeta potentials of CaP, ZnCaP as well as alginate coated ZnCaP (ZnCaP-4) at different pHs are shown in Fig. S4. The calcium phosphate nanoparticles had a high negative zeta potential of −28 mV at a pH of 7. This was increased to −13 mV (at pH 7) after functionalizing with positively charged zinc ions. Alginate coated nanoparticles (ZnCaP-4) exhibited a still more zeta potential at −11 mV at neutral pH.

3.4. Insulin loading and in vitro release

The insulin loading was found to be 28.50 ± 2.12, 29.03 ± 2.26 and 27.35 ± 2.33 IU/100 mg for ZnCaP-1, ZnCaP-2 and ZnCaP-4 respectively. The insulin release profiles in SGF (pH 1.2), SIF (pH 6.8) and PBS (pH 7.4) of three alginate coated particles are shown in Fig. 2. The insulin release in SGF was negligible for all the three formulations. It seems that almost 90% of the drug was released in the first 4 h of the study (in PBS, pH 7.4) for all the formulations. However, insulin in the SIF (pH 6.8) was released slowly with 4% coating giving a more sustained release. Further analysis was done with only 4% alginate coated nanoparticles, ZnCaP-4.

3.5. Raman spectroscopy

The Raman spectra of alginate coated ZnCaP nanoparticles is given in Fig. S5. Several weak absorption bands are visible within 100–300 cm^{-1}. The absorption bands within 400–700 cm^{-1} and 900–1200 cm^{-1} are attributed to PO bending and stretching vibrations, respectively. A strong band at 964 cm^{-1} is attributed to PO stretching. The middle intense band observed at 3400 cm^{-1} is the O–H stretching band. Absorption band at 1412, 1043 and 955 cm^{-1} is attributed to the alginate. Raman spectral chemical mapping of the alginate coated nanoparticles after treatment in different mediums at different timings are shown in Fig. 3. The chemical color images were made by taking the two characteristics peaks; red