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Scalable fabrication of size-controlled chitosan nanoparticles for oral delivery of insulin

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ABSTRACT

Controlled delivery of protein would find diverse therapeutic applications. Formulation of protein nanoparticles by polyelectrolyte complexation between the protein and a natural polymer such as chitosan (CS) is a popular approach. However, the current method of batch-mode mixing faces significant challenges in scaling up while maintaining size control, high uniformity, and high encapsulation efficiency. Here we report a new method, termed flash nanocomplexation (FNC), to fabricate insulin nanoparticles by infusing aqueous solutions of CS, tripolyphosphate (TPP), and insulin under rapid mixing condition ($Re > 1600$) in a multi-inlet vortex mixer. In comparison with the bulk-mixing method, the optimized FNC process produces CS/TPP/insulin nanoparticles with a smaller size (down to 45 nm) and narrower size distribution, higher encapsulation efficiency (up to 90%), and pH-dependent nanoparticle dissolution and insulin release. The CS/TPP/insulin nanoparticles can be lyophilized and reconstituted without loss of activity, and produced at a throughput of 5.1 g h^{-1} when a flow rate of 50 mL min^{-1} is used. Evaluated in a Type I diabetes rat model, the smaller nanoparticles (45 nm and 115 nm) control the blood glucose level through oral administration more effectively than the larger particles (240 nm). This efficient, reproducible and continuous FNC technique is amenable to scale-up in order to address the critical barrier of manufacturing for the translation of protein nanoparticles.

1. Introduction

Polyelectrolyte complex coacervation has been widely used for preparing nanoparticles from ionic polymers [1–3]. This technique is particularly advantageous for encapsulating charged water-soluble therapeutic agents such as proteins and nucleic acids. By tuning the polyelectrolyte characteristics and assembly conditions, complex nanoparticles with a good encapsulation efficiency and retention of bioactivity, particularly for high molecular weight therapeutic agents, could be generated under mild preparation conditions. For example, chitosan (CS) is a naturally occurring polycation that readily complex with nucleic acids to form DNA nanoparticles [4]. It also complexes with other polyanions including heparin, hyaluronic acid, and sodium tripolyphosphate (TPP) to entrap various charged active pharmaceutical ingredients (API) such as insulin. The mixing of the polyelectrolytes is typically achieved through manual mixing, vortex, or drop-wise addition.

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The reproducibility of these methods is poor and the quality of the nanoparticles is often suboptimal with a broad size distribution and non-uniform composition [5–9]. It poses challenges for producing well-controlled nanoparticles to establish an accurate structure-property relationship. It also hinders the scale-up production of nanoparticles for clinical translation.

We have recently developed a new method termed flash nanocomplexation (FNC) to prepare polycation/DNA nanoparticles [10]. This method is adapted from flash nanoprecipitation except that no organic solvent is involved; and the driving force for phase separation is polyelectrolyte complexation rather than solvent exchange. Like flash nanoprecipitation, a confined impinging jet (CIJ) mixer or a multi-inlet vortex mixer (MIVM) is used to facilitate the rapid and efficient mixing of two or more polyelectrolyte solutions via separate streams, thus achieving uniform phase separation and nanoparticle formation [11–16]. This method offers the advantage of *continuous production* of nanoparticles with controlled size ranging about 30–150 nm with a narrow size distribution. In this study, we examine the feasibility of using FNC to encapsulate protein therapeutics using insulin as a model drug with the goal of developing a scalable process for preparing protein nanoparticles with better control in nanoparticle size and size distribution, higher encapsulation efficiency, and better reproducibility.

Insulin is a water-soluble polypeptide with a molecular weight of about 5.8 kDa and a pI of 5.3. Encapsulation of insulin by polyelectrolyte complex nanoparticles is a common approach to protect it from enzymatic degradation and provide a sustained or targeted delivery of insulin [9]. Compared with other delivery methods, oral administration represents the most convenient route. CS/TPP nanoparticles are particularly promising for oral delivery of insulin [5,6,9], given that both CS and TPP are generally regarded as nontoxic and nonirritant materials [7,17]. Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. A wound healing dressings based on chitosan, has been approved by the US Food and Drug Administration (FDA) [18]. The mucoadhesive property and ability to reversibly open up epithelial tight junction (TJ) of CS could potentially improve paracellular transport of insulin or insulin-containing nanoparticles [19]. Due to the charged nature of insulin, it can be readily loaded into CS/TPP nanoparticles without additional chemical crosslinkers or organic solvents [20,21]. Current methods for preparing CS/TPP/insulin nanoparticles involve ionic gelation by gradual drop-wise addition of CS and insulin solution into TPP solution to allow simple bulk mixing. These insulin-loaded CS/TPP nanoparticles made by drop-wise addition or bulk-mixing method are suboptimal in terms of a broad size distribution, low colloidal stability, and poor reproducibility.

In this study, we report the optimization of FNC to prepare CS/TPP/insulin nanoparticles by varying the flow rate, pH and concentrations of the polyelectrolyte streams, and characterize the nanoparticles with respect to size, size distribution, surface charge, encapsulation efficiency, and stability. We also demonstrate nanoparticle size-dependent insulin delivery efficiency using the optimized CS/TPP nanoparticles through oral administration and their efficacy in regulating blood glucose level in a rat model for Type I diabetes.

2. Materials and methods

2.1. Materials

Chitosan (Average M_w = 90 KDa, deacetylation degree = 85%) and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich. Porcine insulin (27.4 IU/mg) was purchased from Wanbang Bio-Chemical Co., Ltd. Fluorescein isothiocyanate (FITC), sulfo-cyanine 5-NHS (Cy5) ester and sulfo-cyanine 7 NHS (Cy7) ester were purchased from Little-PA Sciences Co., Ltd. Occludin antibody was obtained from Gene Tex Inc. Anti-rabbit IgG Fab2 Alexa Fluor(R) 488 Molecular Probes was acquired from Cell Signaling Technology Inc. Porcine insulin ELISA Kit was purchased from Mercodia Inc. Alkaline phosphatase (ALP), aspartate transaminase, (AST), alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (γ-GT) assay kits were purchased from Jiancheng Biotech. Co. Ltd. The hydroxypropyl methyl cellulose phthalate (HPMCP, viscosity: 42.0 cP s) enteric capsules were kindly provided by Shaoxing Kangke Capsule Co., Ltd. All the other reagents were of analytical grade.

2.2. FNC setup for preparation of blank and insulin-loaded CS/TPP nanoparticles

CS was dissolved to a concentration of 1 mg mL^{-1} in double distilled water containing 0.2% acetic acid. The pH of the CS solution was adjusted to 5.3 by 1 M sodium hydroxide (NaOH). TPP was dissolved in HEPES (25 mM) buffer at a concentration of 0.2–0.35 mg mL^{-1}. Insulin was dissolved in hydrochloric acid aqueous solution (pH 2.8) at a concentration of 0.35–0.7 mg mL^{-1}. A four-stream multi-inlet vortex mixer (MIVM) device was constructed based on a reported design (the mixing chamber dimensions and flow setup are illustrated in Fig. 1) [14,15]. TPP solution in HEPES buffer was introduced through Inlet 1, with CS solution in sodium acetate buffer through Inlet 2, insulin solution through Inlet 3, and distilled water through Inlet 4. For preparing empty nanoparticles, Inlet 3 was used to flow buffer solution without insulin. All inlets were running at the same volumetric flow rates controlled by a digital programmable syringe pump.

2.3. Characterization of nanoparticles

The dynamic light scattering (DLS) measurements (for size and polydispersity index, PDI) and zeta potential measurements (for surface charge density) of the nanoparticles were conducted in the preparation medium (9 mM sodium acetate buffer, pH as specified in each experiment) or 10 mM phosphate buffer solution (PBS, pH 6.5) using a Malvern Zetasizer Nano ZS at 25 °C in triplicates. For latter, the nanoparticles were purified by ultrafiltration, and resuspended in PBS (10 mM, pH 6.5). Morphology and size distribution were characterized by transmission electron microscope (TEM). In order to evaluate the encapsulation efficiency (hereafter EE) and loading content (hereafter LC) of CS/TPP/insulin nanoparticles, the nanoparticle suspension was pipetted into the ultrafiltration tube (molecular weight cutoff at 100 kDa), followed by centrifugation at 300 ×g for 20 min. The insulin concentration in the filtrate was examined by measuring absorbance at a wavelength of 280 nm with UV–visible spectrophotometer. EE and LC in percentages were calculated using Equation (1) and Equation (2): [6].

$$EE(\%) = \left(1 - \frac{Amount\ of\ unencapsulated\ insulin}{Total\ amount\ of\ insulin\ added}\right) \times 100\% \quad (1)$$

$$LC(\%) = \frac{Total\ weight\ of\ encapsulated\ insulin}{Total\ weight\ of\ nanoparticles} \times 100\% \quad (2)$$

2.4. Experimental design to test effect of physical parameters on nanoparticles characteristics

2.4.1. Optimization of volumetric flow rate

A series of volumetric flow rates (1–50 mL min^{-1} for each stream) were tested. In fluid mechanics, Reynolds number (*Re*) is a