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Colloidal carrier integrating biomaterials for oral insulin delivery: Influence of component formulation on physicochemical and biological parameters

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Abstract

Strategies to design effective and safe colloidal carriers for biopharmaceuticals have evolved through applying the knowledge gained in nanotechnology to medicine. Designing a colloidal carrier to serve as a protein delivery device requires an understanding of the effect of different materials on the physicochemical, physiological and toxicological parameters for clinical application. The purpose of this study was to evaluate the influence of formulation components on the physicochemical factors and biological function involved in the development and optimization of newly designed nanoparticles for orally dosed insulin. Biodegradable, biocompatible, mucoadhesive and protease-protective biomaterials were combined through ionotropic pre-gelation and polyelectrolyte complexation forming an alginate, dextran sulfate and poloxamer hydrogel containing insulin, stabilized in nanoparticles with chitosan and poly(ethyleneglycol) and coated with albumin. Nanoparticles ranged in size from 200 to 500 nm with 70–90% insulin entrapment efficiency, and electrostatic stabilization was suggested by zeta potential values lower than −30 mV. This combination of formulation components was selected for insulin protection against harsh gastric pH and proteolytic conditions, and to improve insulin absorption through intestinal mucosa by combining nanoparticle uptake and insulin release at the site of absorption. Insulin was shown to be bioactive after nanoparticle formulation and release in neutral pH conditions. Fourier transform infrared spectroscopy was used to confirm the presence of formulations components in the nanoparticle structure and to identify potential interactions between biomaterials.

Keywords: Colloidal carriers; Biomaterials; Insulin; Oral delivery; Development

1. Introduction

Colloidal carriers for orally dosed insulin are of considerable interest in the pharmaceutical sciences, especially for the treatment of diabetes mellitus. The carriers are designed to improve limited absorption of insulin through the gastrointestinal (GI) tract by facilitating its uptake and translocation either by carrier absorption or by increasing the residence time of insulin at the site of absorption during release in the intestinal lumen, while providing protection from acidic and enzymatic degradation [1–3].

Strategies for design and development of nanoparticles as colloidal carriers have evolved through advances in nanotechnology and have focused on incorporating biomaterials and multifunctional polymers as these possess biocompatible, biodegradable, hydrophilic and protective characteristics that can improve insulin absorption through the GI tract due to the effects on particle size, stability, release profile and residence time at the site of absorption [4–6].

One such biomaterial is alginate composed of (1,4)-linked β-D-mannuronic acid and α-L-guluronic acid

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anionic chains with mucoadhesive, biodegradable and biocompatible properties. Alginate forms stable and reversible hydrogel in the presence of multivalent cations due to intramolecular and intermolecular cross-linking, resulting in compact coiled chains of a pre-gel state. The low concentration of multivalent cations interacts selectively with guluronic residues on the same alginate chain, and the intermolecular connections form small alginate nuclei which are stabilized into nanoparticles after polyelectrolyte complexation with polyamines [7,8]. Chitosan is an unbranched polyamine of D-glucosamine and *N*-acetyl glucosamine molecules with mucoadhesive, biodegradable and biocompatible properties. Chitosan stabilizes the alginate nucleus and demonstrates pH-sensitive behavior opposite to that of alginate, being soluble at low pH due to protonation of amino groups and insoluble at higher pH values [9,10]. Chitosan has significant potential to reduce transepithelial electrical resistance and transiently opens the tight junctions between epithelial cells to enhance insulin absorption through the paracellular pathway [11]. The acid type used to dissolve chitosan additionally determines the physicochemical properties of nanoparticles by influencing the stability, strength, and mucoadhesive and disintegrating properties [12,13]. Chitosan dissolved in lactic acid presents greater bioadhesive and flexible elastic properties than chitosan dissolved in acetic acid. Higher strength was reported by using acetic acid due to the influence on intramolecular and intermolecular interactions of chitosan chains [12,14]. Alginate and chitosan nanoparticles have been widely used and extensively reported for oral delivery of insulin [15–17]. The incorporation of highly charged polyanions into chitosan and alginate nanoparticles has been reported to improve insulin entrapment efficiency, and the incorporation of additional polyanions is designed to modulate the retention/release profile by enhancing electrostatic interactions with insulin [18,19]. Dextran sulfate is a negatively charged polymer with biodegradable and biocompatible properties; it contains approximately 2.3 sulfate groups per glycosyl residue and these enhance the negatively charged network.

Stabilizers are important for modulating the structural properties of nanoparticles with a strong tendency to aggregate by providing stability that depends to a great extent on the electrostatic state, and influencing interactions with enzymes and biological membranes. Therefore, absence of electrostatic and steric stabilization explains particle aggregation, enzymatic degradation and rapid clearance of nanoparticles from the GI tract [20]. Poly(ethyleneglycol) (PEG) is a polyether with steric stabilization properties which can reduce the interaction between particles and enzymes of the digestive fluids, and possesses mucoadhesive properties related to chain penetration through the intestinal mucosa for modulating permeation [21,22]. PEG is adsorbed onto the nanoparticle surface through intermolecular hydrogen bonding between the electropositive amino hydrogen of chitosan and the electronegative oxygen atom of PEG, thus forming a chitosan–PEG semi-interpenetrating network [23]. Poloxamer is a non-ionic triblock copolymer composed of a central hydrophobic chain of poly(oxypropylene) (PPO) and two hydrophilic chains of poly(ethylene oxide) (PEO) with steric stabilization properties due to its amphiphilic structure. Poloxamer is bound to the nanoparticle surface possibly by hydrophobic interaction of the PPO chains with methyl groups of chitosan, while the hydrophilic PEO chains protrude into the surrounding medium to create a steric barrier [24].

Protection against enzymatic degradation represents an essential strategy for oral delivery of insulin. Factors that counteract the pharmacological effect of orally dosed insulin were calculated as 60% due to insulin degradation, 23% to premature insulin release and 17% to lack of mucoadhesion [25]. Based on these factors, albumin coating is designed to act as a sacrificial target to avoid insulin degradation by preventing proteases from accessing insulin within the nanoparticle and by stabilizing the nanoparticle in the acidic environment of the stomach [26,27].

The design of these nanoparticles can thus be described as a multilayer complex retaining insulin within the nanoparticle consisting of calcium cross-linked alginate and dextran sulfate nucleus followed by a layer of chitosan and stabilizer (poloxamer/PEG) and an outermost coating of albumin.

The purpose of this study was to evaluate the influence of calcium chloride and the addition of dextran sulfate, poloxamer, PEG and albumin on physicochemical and biological parameters involved in optimization of alginate–chitosan nanoparticles for oral delivery of insulin. Physicochemical factors were analyzed in terms of reducing particle size to increase nanoparticle uptake, obtaining stable nanoparticles in suspension, increasing insulin entrapment efficiency to increase process efficiency and controlling insulin release in simulated digestive fluids. In vitro insulin bioactivity was evaluated as a biological parameter to determine the influence on insulin activity after nanoparticle formulation. Fourier transform infrared spectroscopy (FTIR) was used to confirm the presence of various formulation components in the nanoparticle structure, and to determine interactions between alginate and chitosan, and between chitosan and dextran sulfate and albumin.

2. Materials and methods

2.1. Materials

Alginic acid sodium salt from brown algae, low molecular weight chitosan (50 kDa), bovine serum albumin (BSA) and trifluoroacetic acid (TFA) 99% were purchased from Sigma–Aldrich Chemie (France); dextran sulfate sodium salt from *Leuconostoc* ssp., ethylenediaminetetracetic acid disodium salt dihydrate (EDTA) and poly(vinylpyrrolidone) K 30 (PVP K30) were purchased from Fluka (Switzerland); poloxamer 188 (Lutrol F68) was kindly supplied

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