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PEGylated starch acetate nanoparticles and its potential use for oral insulin delivery

P.F. Minimol, Willi Paul, Chandra P. Sharma*

Division of Biosurface Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Poojappura, Thiruvananthapuram 695012, India

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ABSTRACT

A novel controlled release formulation has been developed with PEGylated starch acetate nanoparticles. Biodegradable polymers, such as starch, have been studied for various pharmaceutical applications because of their biocompatibility and biodegradability. Starch acetate is one of the hydrophobic biodegradable polymers currently being used or studied for controlled drug delivery. Polyethylene glycol was conjugated with starch acetate, to obtain an amphiphilic polymeric derivative. On its incubation with insulin solution at the critical micelle concentration, self-aggregated nanoparticles with mean particle size of 32 nm are formed. These self-aggregated nanoparticles with associated insulin have enhanced encapsulation efficiency. The mean particle size of these nanoparticles increased with the increase in the molecular weight of PEG. Present study indicated that PEGylated starch acetate nanoparticles are highly bioadhesive and can be utilized as a carrier system for controlled delivery of insulin or other proteins for various therapeutic applications.

1. Introduction

Starch is one of the better known natural and biodegradable biopolymers. Starch and its derivatives received much attention in the food, plastic and pharmaceutical industries because of its gelling, film forming and biodegradable properties (Ogura, 2004). The major drawback of starch in use as a controlled release agent is its hydrophilicity (Michailova, Titeva, Kotsilkova, Krusteva & Minkov, 2001). During the last decade, new generation polymeric materials have been investigated for drug delivery applications. Different modifications or derivatization techniques have been tried for developing new polymers from natural biopolymers. One approach is acetylation of starch which converts the hydrophilic starch to hydrophobic starch acetate. Starch acetate has been widely used for drug delivery applications (Korhonen, Kanerva, Vidgren, Urtti & Ketolainen, 2004; Nutan, Soliman, Taha & Khan, 2005; Nutan, Vaithiyalingam & Khan, 2007; Pajander, Soikkeli, Korhonen, Forbes & Ketolainen, 2008; Pohja, Suihko, Vidgren, Paronen & Ketolainen, 2004; Pu, Chen, Li, Xie, Yu & Li, 2011; Tuovinen, Peltonen, et al., 2004; Tuovinen, Ruhanen, et al., 2004; van Veen et al., 2005; Xu, Yang & Yang, 2009) and has also been investigated as tissue engineered scaffold (Guan & Hanna, 2004; Reddy & Yang, 2009).

Acetylation of starch has received much attention for varied drug delivery applications, such as in the preparation of coatings for sustained release of drugs. Hydrophobically modified polymers have attracted much attention in drug delivery applications due to their subtle balance of hydrophilic–hydrophobic nature and biodegradability (Daoud-Mahammed et al., 2007; Wintgens & Amiel, 2005). They spontaneously self-associate forming hydrophobic cores with considerable potential in drug/gene delivery research and other biomedical applications (Couillet, Hughes & Maitland, 2005; Nilsson, Thuresson, Lindman & Nyström, 2000). This self-association does not allow fluids to penetrate into the particles thereby protecting the encapsulated proteins from the proteolytic enzymes in the intestine. These nanoparticles are utilized for drug delivery applications because of their high stability, prolonged residence time, high drug encapsulation, better storage life and the ability to translocate through the intestinal barrier; by the paracellular pathway or via M cells in Peyer's patches (Hussain, Jaitley & Florence, 2001). Polymeric micelles are systems constituted by self-aggregation of graft-copolymers bearing both hydrophilic and hydrophobic chains (amphiphilic polymers). In aqueous medium at a proper concentration (critical aggregation concentration, CAC) these copolymers aggregate, forming colloidal micellar systems having a hydrophobic core and a hydrophilic shell (Nagarajan, 2011). Active molecules can be either covalently or physically linked to amphiphilic copolymers, and in the aggregation process remain incorporated in the hydrophobic core. The dissociation process of these colloids and the hydrolysis of

* Corresponding author. Tel.: +91 471 2520214; fax: +91 471 2341814.
E-mail address: sharmacp@sctimst.ac.in (C.P. Sharma).

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subsequent chemical linkages allow the release of the active substances (Kim, Cha & Ahn, 2010). It is hypothesized that hydrophobic starch acetate on reaction with polyethylene glycol can self-aggregate, in the same way, to form a hydrophobic core protecting the drug inside. Therefore, an attempt has been made to develop and characterize self-aggregating nanoparticles from PEGylated starch acetate for possible approach toward orally delivering insulin.

2. Materials and methods

Starch (soluble, ACS reagent, MW 342) and *O,O′*bis(2-aminopropyl)poly(ethylene glycol) with molecular weights of 1900, 800 and 500 were obtained from Sigma Chemical Co., USA. Acetic anhydride and dicyclohexylcarbodiimide (DCC) were from Merck KGaA, Darmstadt, Germany. Insulin (human, 400 IU/ml) was a gift from USV Ltd., Mumbai, India. ZO-1 Antibody (H-300) used for the detection of ZO-1 was from Santa Cruz Biotechnology Inc., USA (sc-10804). All other chemicals and solvents used were of analytical reagent grade.

2.1. Preparation of starch acetate

Starch was allowed to react with acetic anhydride (1:4 ratio) with sodium hydroxide as a catalyst as per the reported method (Xu, Miladinov & Hanna, 2004). Briefly, 12 g of starch was suspended in 40 ml of acetic anhydride in a round bottom flask. This was allowed to react for 30 min, and 1.5 ml of 50% sodium hydroxide was added as a catalyst. The temperature was then rapidly increased to 80 °C, and maintained for 20 h, to obtain a dark brown viscous solution. The reaction was quenched by the addition of excess ice-cold water to the viscous solution. The starch acetate precipitate was collected by filtration and washed with distilled water. Washing was continued till the NaOH traces and the brown color were completely removed. This was monitored visually and by noting the pH of the washings. The white starch acetate precipitate was then dried in an oven for 24 h at 37 °C.

2.2. PEGylation of starch acetate

Starch acetate (0.5 g) was dissolved in 20 ml of acetone. 100 mg of dicyclohexylcarbodiimide (DCC) was added to this solution and allowed to react for 1 h for the activation of OH group. 0.5 g PEG dissolved in 3 ml acetone was added to the activated starch acetate solution and allowed to react for 24 h at room temperature. After 24 h, the solution was precipitated in water and washed thoroughly. These particles were dissolved in acetone and re-precipitated in water. The precipitate was freeze dried, at −40 °C, to obtain PEGylated starch acetate.

2.3. Characterization of PEGylated starch acetate

The FTIR spectra of the starch acetate and PEGylated starch acetate were obtained from Perkin–Elmer Paragon 1000 FTIR spectrometer (KBr pellets), with a resolution of 4 cm^{-1} between 500 and 4000 cm^{-1}. A ^1H NMR analysis was performed on PEGylated starch acetate in D_2O using a 500 MHz spectrometer (Bruker Avance DPX 300). Elemental (CHN) analysis of the starch acetate, activated starch acetate and PEGylated starch acetate was done using Perkin Elmer 2400 Series II CHNS/O analyzer. The particle size and zeta potential of these self-aggregated nanoparticles were analyzed by photon correlation spectroscopy and laser Doppler anemometry, respectively, with a Zetasizer, Nano ZS and the pH titrations with MPT-2 autotitrator (Malvern Instruments Limited, UK) at 25 °C.

2.4. Critical aggregation concentration (CAC) of SA-PEG nanoparticles

PEGylated starch acetate was dissolved in acetone. This solution (0.2 ml) was added to water or insulin solution, to form micelle in water or insulin solution. The measurements were made with different concentrations of PEGylated starch acetate. The intensity of scattered light was monitored using a Zetasizer, Nano ZS (Malvern Instruments).

2.5. Preparation of self-aggregated nanoparticles and association of insulin

A solution of PEGylated starch acetate in acetone with a concentration of 0.017 mg/ml was prepared. This was added to the insulin solution (400 IU/ml) to obtain insulin loaded self aggregated nanoparticles. The nanoparticle suspension was centrifuged at 9844 × *g* for 10 min, and the pellet obtained was dried in a refrigerator at 4 °C. Encapsulation efficiency and drug loading of the nanoparticles were evaluated. A known amount of drug loaded nanoparticles was incubated at 37 °C in a known amount of phosphate buffer (pH 7.4) for 24 h. This was filtered using a 0.4 micron syringe filter and the protein content estimated by Lowry's method at 750 nm using UV spectrophotometer (UV l60A, Shimadzu). Nanoparticles formed by the addition of starch acetate to insulin solution were removed by centrifugation, and the supernatant protein was analyzed for its content. The encapsulation efficiency (EE) and loading content (LC) were calculated using the following formulas.

$$EE(\%) = (C_i - C_f)/C_i \times 100$$

$$LC(IU/mg) = (C_i - C_f)/W$$

where C_i and C_f is the initial and final protein concentration respectively and W the dry weight of nanoparticles formed.

2.6. Mucoadhesiveness of PEGylated starch acetate nanoparticles

Mucoadhesion testing of the nanoparticles was carried out using a texture analyzer (TA XT plus, Stable Micro Systems, UK) with 50 N load cell equipped with a mucoadhesive holder. Nanoparticles were attached to the cylindrical probe (10 mm in diameter) by double-sided adhesive tape. Rat intestinal tissue (about 20 × 20 mm) was equilibrated for 15 min at 37 °C before placing it onto the holder stage of a mucoadhesive holder and maintained at 37 °C during the test in buffer medium. The hydrated nanoparticles attached to the probe were then moved downward to make contact with soaked tissue at a contact force of 0.05 N. This was maintained for a period of 1 s. The probe was subsequently withdrawn at a speed of 0.5 mm/s. By using the texture analyzer, the maximum force required to separate the probe from the tissue (i.e. maximum detachment force; F_{max}) could be detected directly from Texture Exponent 32 software, and the total amount of forces involved in the probe withdrawal from the tissue (work of adhesion; W_{ad}) was then calculated from the area under the force versus distance curve. These parameters were used to compare the different test conditions or formulations.

2.7. Cytotoxicity of PEGylated starch acetate nanoparticles

The *in vitro* cytotoxicity of the nanoparticles was evaluated by MTT assay (Mosmann, 1983) done on mouse fibroblast (L929) cell lines as per the directions of ISO standard (Organisation, 2009). The cells were cultured in MEM supplemented with 10% fetal bovine serum and kept at 37 °C in a humidified atmosphere of 5% CO_2. Cells