Journal of Molecular and Genetic Medicine

Saboktakin et al., J Mol Genet Med 2015, 9:2
DOI: 10.4172/1747-0862.1000170

Research Article Open Access

pH Sensitive Chitosan-based Supramolecular Gel for Oral Drug Delivery of Insulin

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Received date: April 11, 2015; **Accepted date:** May 05, 2015; **Published date:** May 12, 2015

Abstract

The main objective of this study is development of chitosan supramolecular gels to utilize for oral delivery of insulin. Chitosan (CS) blended dextran sulfate (DS) matrix as nanogels were characterized by scanning electron microscopy and FT-IR. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). Insulin entrapped in these gels and in vitro release profiles were established. In vitro release of insulin was also evaluated. The CS-DS gels have been developed based on the modulation of ratio show promise as a system for controlled delivery of the insulin to the stomach.

Keywords: Chitosan; Dextran sulfate; Drug delivery; Supramolecular gel; Insulin

Introduction

Hydrogel-based drug delivery systems are of interest due to their attractive characteristics, which can lead to targeting delivery, extension of circulation time, and reduction of toxicity and side effects. Particularly, hydrogels that can be formed in situ under physiological conditions have recently been paid much attention as promising drug carriers [1]. Many hydrogels have been developed that can maintain drug levels within the narrow concentration window by controlled release, which is essential to avoid toxicity due to overdose or ineffectiveness from under dosing. Furthermore, hydrogel based controlled drug delivery systems can avoid frequent or continuous administrations which is beneficial to patients' convenience and comfort [2,3]. However, current hydrogels as drug delivery tools are limited by their hydrophilic nature, which prevents their use as drug delivery systems for hydrophobic drugs because of a rapid drug release in the initial phase. Furthermore, because of the hydrophobic interactions, large drug aggregates may be formed during the drug loading process, which can result in a high local concentration and cause side effects or even toxicity [4-8]. To achieve a hydrogel based controlled delivery systems for hydrophobic drugs, we have introduced ß-cyclodextrin or human serum albumin to the hydrogel matrix as a primary accommodation site for those hydrophobic molecules and in vitro controlled release of model hydrophobic drugs were observed [9]. Although positive preliminary in vivo results have been obtained by using zebra fish embryos as the animal models, further systematic in vivo studies have to be performed by using different mammalian animals before the clinical trials. In the past few years, a number of therapeutic proteins have been developed against a broad range of diseases such as cancers, autoimmune diseases and metabolic disorders [10,11]. However, most of these effective therapeutic proteins are prevented from clinical use by fundamental technical hurdles particularly with regard to delivery. Hydrogels are an ideal candidate for protein delivery, as they contain large amounts of water in the polymer network in a way similar to body tissues. Thus it allows retaining the proteins in the protective 3-D network in their active forms and prevents them from denaturation during administrations. Sustained delivery of proteins using hydrogel systems have been reported, in which the loaded proteins are released from the hydrogel matrix in a time dependent manner. However, a triggerable delivery system for proteins which enables an on-demand controlled release profile might enhance its therapeutic effectiveness and reduce systemic toxicity [12-16]. Recent advances in nanotechnology have spurred developments in hydrogel based drug delivery systems, nanosized hydrogels (nanogels) have attracted considerable attention, which offers the possibilities of intracellular delivery and administration by intravenous injection. Furthermore, nanogel particles can maintain the properties of their hydrogel counterparts but with a largely increased surface area and controlled sizes or shapes, which can contribute to improving efficiency of drug delivery to tumors [17-19]. Chitosan-based nanogels have been studied as carriers of oligonucleotides in gene therapy, for mucosal vaccination, in tissue engineering or drug delivery. However, highly positive nanogels are generally cytotoxic, or are unselectively taken up by cells due to their positive charge, suggesting that developing negative chitosan-based nanogels could be of biological interest [20]. Nanogels based on a chitosan core matrix decorated on their surface with polyanions have been described. In this study our aim was to utilize the pH sensivity of nanogel, which can be used for protecting insulin in stomach and bioadhesivity of chitosan to make prolonged contact with the intestinal mucosae, so as to increase the absorption of insulin. Insulin was entrapped in these gels and the in vitro release profiles and stability of insulin in contact with these nanogels during the release were studied [21,22].

Materials and Methods

Materials

The chitosan (medium MW 400,000 Da, 85% deacetylation) and sodium dextran sulfate (MW 12,750 Da) were purchased from Fluka/

Sigma-Aldrich. Chitosan blended dextran sulfate matrix was prepared by the methods described in the literatures and then dried by freeze drying method. The insulin used was recombinant human insulin. All the other chemicals used were of analytical reagent grade. Enzyme-free SGF (pH=1) or SIF (pH= 7.4) were prepared according to the method described in the literature [21].

Instruments

Melting points were obtained on a Mel-Temp melting point apparatus. Analytical TLCs were run on commercial Merck plates coated with silica gel GF250 (0.25 mm thick). The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali, using a 1 cm quartz cell. The nanogels samples were obtained by Freeze dryer Model FD-10. The samples were examined to determine the mean diameter and size distribution. The powder morphology hydrogel in the form of pellets(to measure grain size) was investigated using Philips XL-30 E SEM scanning electron microscope (SEM) at 30 kv (max.). The samples were prepared by physical vapor disposition method. The gold layer thickness was about 100 A˚ at these samples.

Preparation of CS–DS hydrogel

CS-DS hydrogels were prepared by the complex coacervation of CS and DS. CS (0.25%) and DS (1%) (wt/vol) solution were prepared by dissolving CS in aqueous acetic acid or DS in water. The concentration of acetic acid was kept 1.75 times higher than that of CS in all cases to maintain the CS in the solution. The solution was then mixed with 5 mL of respective concentration of the CS solution under magnetic stirring (~ 200 rpm) at room temperature. The nanogels / microgels suspension was formed spontaneously. The mixture was stirred for a further 15 minutes. Both the pH and the particle size of the nanogels suspension were measured.

Insulin loading in hydrogel

Insulin can only be dissolved in acidic aqueous solutions of around pH 3.0, insulin was first dissolved at the pH 3.0 and then the pH was increased to pH 7.2 using 0.1M KOH. Subsequently, 10 mg of hydrogel was placed in 3 mL of insulin solution (1.0 mg/mL) to absorb the total amount of the insulin solution. After approximately 60 min., the completely swollen hydrogel loaded with insulin were placed in desiccators and dried under vacuum at room temperature.

Scanning electron microscopy

Surface and shape characteristics of CS-DS hydrogels were evaluated by means of a scanning electron microscope (FEI-Qunta-200 SEM, FEI Company, Hillsboro, OR). The samples for SEM were prepared by lightly sprinkling the hydrogel on a bouble adhesive tape, which stuck to an aluminum stub. The stubs were than coated with gold to a thickness of ~300 Å using a sputter coater and viewed under the scanning electron microscope.

Quantitative analysis of insulin

Hydrogel (3 mg) adduct was dispersed in 3 mL of mobile phase solution. The reaction mixture was maintained at 37°C. After 4 h the hydrolysis solution filtered and analyzed by HPLC for the determination of total insulin in hydrogels.

Insulin stability during release studies from hydrogel

In order to study the stability of insulin in contact with hydrogel, two different conditions were chosen: 37°C and darkness, 37°C and light. Insulin was loaded in hydrogel and then the peptide stability was investigated during release under the above mentioned conditions at two different pH values of 1 and 7.4. Samples were analyzed under each condition after 24 and 48 h. In this condition insulin remained fairly stable at both pH values during the course of experiments, indicating that adsorption of the peptide too the hydrogel and their release afterwards did not substantially influence the stability of this peptide. To investigate the protective ability of the hydrogel for insulin in the harsh environment of the stomach, insulin and insulin-incorporated were treated with a simulated gastric solution that contained endopesidase pepsin.

Particle size

After drying at 37°C for 48 h, the mean diameter of the dried hydrogel was determined by a sieving method using USP standard sieves. Observations are recorded in Table 1.

Formulation Drug (wt% of polymer)	Encapsulation Efficiency (%)	Mean Diameter (nm)	Poly-dispersity Index
Blank	-	84.32 ± 25.0	0.24 ± 0.01
10	54.50 ± 0.52	92.21 ± 15.0	0.32 ± 0.05
20	62.12 ± 3.6	105.58 ± 40.2	0.68 ± 0.01
30	89.20 ± 4.0	156.92 ± 40.5	0.84 ± 0.10

Table 1: Characterization of blank and insulin loaded CS – DS hydrogel.

Equilibrium swelling studies

The water absorbency of CS-DS hydrogel was investigated in different pH buffer solutions (pH 4.0, 7.4, and 10.0). CS-DS hydrogel specimens of a known weight (W0) were immersed in buffer solutions at 37°C. The swollen hydrogel was taken out from the buffer solution at predetermined intervals and weighed (Wt) after removing excess solution from the surface with a wet filter paper. The swelling ratio was recorded as Q=Wt / W0 where W0=the weight of the hydrogel at time zero and Wt=the weight of hydrated hydrogel at time t. Experiments were performed in triplicate.

Results and Discussion

Morphology of CS–DS hydrogel

The insulin loaded CS-DS hydrogels were characterized by FESEM for their size and distribution. Data showed that hydrogel have a solid and near consistent structure. These hydrogels have good spherical geometry. Figure 1a and b show the Morphology of insulin loaded CS-DS hydrogels by emission scanning electron microscopy field and the size distribution of insulin loaded CS-DS hydrogel by the SEM, respectively. Also, the results show that the surface of CS-DS hydrogel shrank and a densely cross-linked gel structure was formed. The retardation of drug release from matrices of higher crosslinker content.

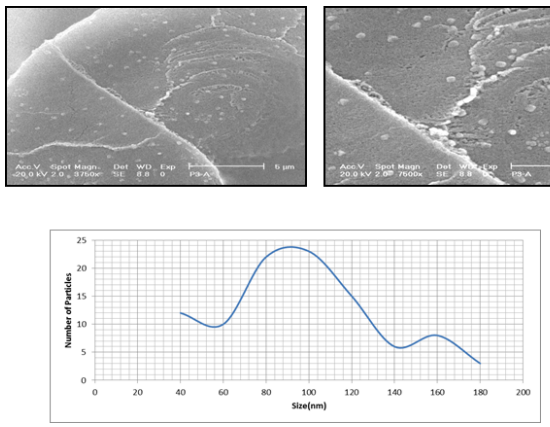

Figure 1: a) Morphology of insulin loaded CS-DS hydrogels by emission scanning electron microscopy field, b) the size distribution of insulin loaded CS-DS hydrogel was determined from the SEM picture.

The size of CS-DS hydrogels was estimated by scanning electron microscopy (SEM) of the dried hydrogel dispersions. The determination of hydrogel size by SEM under a dry state does not result in an accurate absolute value of the hydrated hydrogel size in dispersion, but only visualizes size range and particle shape. The SEM picture of insulin loaded-hydrogels is shown in Figure 1b. SEM analyses confirmed the relatively broad size distribution of the CS-DS hydrogels, ranging from about 40-100 nm. The incorporation of insulin into the CS-DS hydrogel produced a smooth surface and compact structure. The CS-DS hydrogels are hydrophilic and would be expected to swell in water, thus producing a large hydrodynamic size when measured by the Zetasizer.

In vitro release studies

To develop potential applications of polymer-bonded drugs (PBDs) containing of insulin as the pharmaceutically active compound. We studied the hydrolysis behavior of hydrogel polymers under physiological conditions. Although the polymers were not soluble in water, they were dispersed in a buffer solution, and the hydrolysis was evaluated as a heterogeneous system. Figure 2 show the release of insulin from nanogel as a function of time at 37°C in pH 1.0 and pH 7.4. The degree of hydrolysis of hydrogels containing of insulin as a function of time is shown in Figures 2 and 3. Nano polymer loaded insulin (50 mg) was poured into 3 mL of aqueous buffer solution (pH 7.4) (Figure 2). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37°C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The triplicate samples were analyzed by UV spectrophotometer, and the quantity of insulin were determined using a standard calibration curve obtained under the same conditions. The primary mechanism for release of insulin from matrix systems in vitro are swelling, diffusion, and disintegration (Figure 3). In vitro degradation of CS-DS hydrogels were prepared by solution casing method occurred less rapidly as the degree 73% deacetylated showed slower biodegradation. Since the grade of CS-DS used in the present study was of high molecular weight with a degree of deacetylation ≥85%, significant retardation of release of insulin from bead is attributed to the polymer characteristics. In addition, diffusion of insulin may have been hindered by increased tortuosity of polymer accompanied by a swelling mechanism.

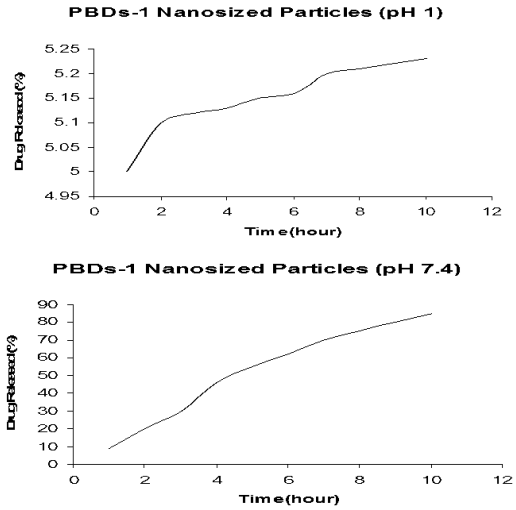

Figure 2: Release of insulin from nanogel as a function of time at 37°C in pH 1.0 and pH.

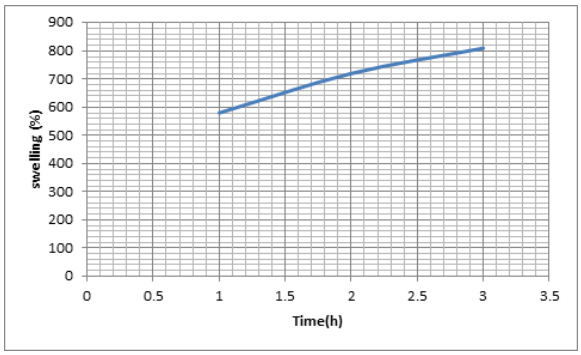

Figure 3: Time-dependent swelling behavior of insulin loaded CS-DS hydrogel at 37°C.

Insulin – loaded CS-DS hydrogels were obtained spontaneously upon the mixing of the DS aqueous solution (0.1% wt/vol) with the CS

solution (0.1% wt/vol) under magnetic stirring, with insulin dissolved in CS-DS solution. The incorporation of insulin into the CS-DS hydrogels resulted in a sharp increase in the particle size of the hydrogels dispersion. The significant increases in particle size give a good induction of the incorporation of insulin into CS-DS hydrogels. The transport of insulin nanoparticles indicated an enhancement role exerted by the nanoencapsulation in the polymeric matrix with reference to insulin alone used as control.

Conclusions

The important conditions for colon-selective drug delivery, a polymer are needed that is able to withstand lower pH values, but disintegrates at the slightly alkaline pH values of the ileocecal junction and the large intestine. pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. The hydrolysis of insulin-polymer conjugates were performed at pH 1 and 7.4 at 37°C. The different systems available with varied release kinetics may help in tailoring the system suitable for the oral delivery of insulin for the management of diabetes.

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