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Controlled delivery of oral insulin aspart using pH-responsive alginate/κ-carrageenan composite hydrogel beads

Hui-Peng Lim[a], Chien-Wei Ooi[a,b], Beng-Ti Tey[a,b], Eng-Seng Chan[a,b,*]

[a] Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Selangor, Malaysia
[b] Advanced Engineering Platform, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Selangor, Malaysia

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ABSTRACT

Diabetes mellitus is a global epidemic currently affecting > 415 million people worldwide. It is a disease caused by either the lack of or a resistance to the insulin, which is a glucose-regulating hormone, in a patient. The low compliance with the subcutaneous administration of insulin by diabetic patients has urged the need for an oral-route delivery of insulin. There are two important criteria for an effective oral delivery of insulin, namely the protection of encapsulated insulin from the harsh acidic conditions in the stomach, and the controlled release of insulin at the targeted site of absorption (i.e., the intestine). In this work, the pH-responsive composite hydrogel beads made of the naturally-derived biopolymers (i.e., alginate and κ-carrageenan) were formed using the extrusion-dripping method. The composite hydrogel beads were tested as the delivery vehicles for insulin aspart. At pH 1.2, the composite hydrogel beads successfully retained the insulin aspart through electrostatic interaction between the positively charged insulin aspart and the negatively charged sulfate groups of the κ-carrageenan polymers. At pH 7.4, insulin aspart was released in a gradual manner, and the release profile approached zero-order kinetic when the concentration of κ-carrageenan used in the formation of hydrogel bead increased. After incubation of composite hydrogel beads in acidic simulated gastric medium, there was approximately 65% of the insulin aspart remained biologically active in the beads. The results suggest that the alginate/κ-carrageenan composite hydrogel bead is a promising delivery system for the oral insulin aspart.

1. Introduction

Insulin has been the mainstay of treatment for patients with type 1 (insulin-dependent) or advanced type 2 (non-insulin-dependent) diabetes [1] since its first discovery in the canine pancreas by Banting and Best in 1921 [3]. Today, a variety of regular and analog insulin formulations are available in the market. Insulin aspart, which is an analog insulin manufactured by Novo Nordisk [29], has an absorption rate twice as fast as human insulin and has a significantly lower risk of hypoglycemia in diabetic patients [11,17]. Nevertheless, till date, the common route of administration for exogenous insulin is parenteral. This route of administration has several drawbacks. For instance, the needle pricking process causes considerable discomfort to the patient, and the multiple injections required daily also create inconvenience to the patient. In addition, the occasional hypoglycemia due to the insulin overdose and the risk of infection at the injection sites further lead to a poor adherence to medication [52], which consequently jeopardizes the efficient treatment for the diabetic patients.

Oral administration, which has a high level of acceptance by patient, thus becomes a highly desirable alternative. While clinical trials are underway for several oral insulin products [12], an intense research effort is still required for the development of a robust oral insulin delivery system that could replace the injection-based insulin delivery in near future. One of the major hurdles is that the insulin is prone to acid-catalyzed degradation in stomach [7] before reaching the targeted site of absorption, which is the intestine. Therefore, the polymeric encapsulation of insulin is viewed as a promising method to protect the insulin from the harsh acidic environment in the stomach and to provide controlled release of insulin in the intestine.

In recent years, natural polymers that are generally hydrophilic have received much attention from researchers due to their advantageous features such as low cost, renewability, biodegradability, biocompatibility, and non-toxicity [25]. Some examples of the polymers are psyllium [41,46], gelatin [45], and alginate [14]. Alginate, a polyanionic natural polymer derived from brown seaweed, is composed of α-L-guluronic (G) and (1→4)-linked β-D-mannuronic (M) acid residues. The pK_a values of the M and G acid residues are 3.5 and 4.0, respectively [21]. Alginate is a desirable encapsulation material due to

* Corresponding author at: Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Selangor, Malaysia.
E-mail addresses: lim.hui.peng@monash.edu (H.-P. Lim), ooi.chien.wei@monash.edu (C.-W. Ooi), tey.beng.ti@monash.edu (B.-T. Tey), chan.eng.seng@monash.edu (E.-S. Chan).

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its mild processing conditions (i.e., room temperature and near neutral pH). Furthermore, it has been generally regarded as safe (GRAS) by the Food and Drug Administration (FDA) under Code of Federal Regulations (21 CFR Parts 184). Nevertheless, the inherent porous structure of pure alginate hydrogel is not favorable to the encapsulation of the hydrophilic and low-molecular-weight insulin (i.e., 5.8 kDa) because of the burst release of insulin under gastric pH condition [40,44].

To overcome the aforementioned shortcoming of pure alginate hydrogel, several approaches have been proposed: coating of alginate hydrogel with polycationic chitosan [39,53], and reinforcement of alginate matrices with polymers like chitosan [44], whey protein [10], and dextran sulfate [28,35]. In particular, the alginate beads reinforced with dextran sulfate displayed a better retention of insulin under gastric pH condition, as compared to the alginate beads coated with chitosan [28]. Dextran sulfate is a synthetic sulfated polysaccharide produced from the sulfuric esterification of dextran, which is a polysaccharide produced from sucrose by the dextran-producing bacteria [51]. The synthesis of dextran and the subsequent esterification of dextran to dextran sulfate are laborious, costly, and low in product yield [48]. Furthermore, dextran sulfate has yet to be recognized as a GRAS material.

The aim of this study is to develop a pH-responsive composite hydrogel bead for the oral delivery of insulin aspart. The composite hydrogel bead is comprised of a three dimensional alginate hydrogel matrices reinforced with κ-carrageenan. κ-carrageenan is a naturally occurring sulfated polysaccharide extracted from red marine algae. It is a linear polyanionic polysaccharides composed of sulfated galactose and 3,6-anhydrogalactose copolymers, linked by alternating α-1,3 and β-1,4 glycosidic bonds [4]. It is also a GRAS material (21 CFR Parts 172) widely used in pharmaceutical formulation as the gelling and thickening agent. Since κ-carrageenan is inexpensive as well as safe for consumption, it can be a promising alternative to dextran sulfate. Furthermore, the strong ionization degree of the sulfate pendant groups with low pK_a value (i.e., ~ 2) of κ-carrageenan [24] was hypothesized to be helpful in retaining the encapsulated insulin aspart under acidic pH condition. The effects of κ-carrageenan concentration on the physicochemical and mechanical properties of the alginate/κ-carrageenan composite beads were evaluated. The profiles of insulin aspart released from the hydrogel beads were obtained and subsequently fitted into different kinetic models to reveal the release mechanisms.

2. Experimental

2.1. Materials

A medium-molecular-weight sodium alginate (Manugel GHB, M/G ratio of 0.59) was purchased from FMC Biopolymers, UK. κ-carrageenan was purchased from Sigma-Aldrich, Denmark. Insulin aspart solution (NovoRapid® 100 IU: 3.5 mg/mL, Novo Nordisk, Denmark) was purchased from Sunway Medical Centre Sdn. Bhd., Malaysia, and was used as received. Sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were purchased from Sigma-Aldrich, USA. Acetonitrile (HPLC grade), ortho-phosphoric acid (H_3PO_4, 85%), and sodium sulfate anhydrous (Na_2SO_4) were purchased from Friendemann Schmidt Chemical, Australia. Sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), and hydrochloric acid (HCl) were purchased from Fisher Scientific, UK. Simulated gastric fluid (SGF, pH 1.2) containing 0.01 N HCl and 2 mg/mL NaCl was prepared according to the US Pharmacopeia XVII method [38]. Phosphate buffered saline (PBS, pH 6.8, 10 ×) purchased from Nacalai Tesque, Japan, was diluted ten-fold with water to prepare the simulated intestinal fluid (SIF, pH 7.4). Ultrapure water (Milipore, 18.2 MΩ·cm resistivity) was used throughout the study.

2.2. Methods

2.2.1. Measurement of zeta potential

The zeta potentials of alginate, κ-carrageenan, and insulin aspart were determined as a function of pH. The pH of analyte solution was manually adjusted by 0.1 M HCl or 0.1 M NaOH. The zeta potential was measured using Zetasizer (Nano ZS, Malvern Instruments, UK) at 25 °C. Triplicate readings for each sample were recorded.

2.2.2. Determination of viscosity

The viscosities of κ-carrageenan solution and mixture of alginate/κ-carrageenan/insulin aspart were measured using a rotational viscometer (VM-1B, Heloisfo, Singapore) at room temperature by using a shear rate of 1 s^{-1}. The measurement was done in triplicate.

2.2.3. Preparation of composite hydrogel beads

A stock solution of alginate (4% w/v) was first prepared by dissolving alginate powder in ultrapure water under stirring at 1000 rpm for 3 h. Next, a pre-weighted amount of κ-carrageenan powder was added into the alginate stock solution that had been pre-heated to 55 °C in a water bath. Then, the mixture was subjected to magnetic stirring at 300 rpm for 1 h. The setup was carefully sealed using aluminium foil to prevent water evaporation during heating. The alginate/κ-carrageenan stock solution was brought to room temperature before use. On the other hand, the insulin aspart solution was diluted with an equal volume of water. The diluted insulin aspart solution was then mixed with an equal volume of the alginate/κ-carrageenan stock solution under magnetic stirring at 750 rpm for 30 min. Subsequently, the alginate/κ-carrageenan/insulin aspart solution was debubbled prior to the step of forming composite hydrogel beads. The final concentrations of alginate and insulin aspart were 2% w/v and 0.875 mg/mL, respectively. The final concentration of κ-carrageenan was varied from 0 to 1% w/v.

To prepare the insulin aspart-loaded alginate/κ-carrageenan composite hydrogel beads, 10 mL of alginate/κ-carrageenan/insulin aspart solution was extruded dropwise through a 21-gauge needle tip (inner diameter = 0.51 mm) into 300 mL of gelation bath made of 2% w/v $CaCl_2$ and ultrapure water. The hydrogel beads were allowed to harden for 15 min in the gelation bath before they were harvested. Lastly, the collected hydrogel beads were rinsed extensively using ultrapure water to remove excess Ca^{2+}. The preparation of blank alginate/κ-carrageenan hydrogel beads was in accordance to the afore-mentioned steps, except that the insulin aspart solution was not added.

2.2.4. Characterization of hydrogel beads

2.2.4.1. Size and shape analysis. Thirty units of randomly chosen hydrogel beads were first immersed in a commercial food-colouring dye solution (artificial true blue colour) (Star Brand, FFM Berhad) for 3 min. Next, the stained hydrogel beads were removed from the dye solution using a stainless-steel sieve and were blotted dry. The images of stained hydrogel beads were taken using a digital camera. The images were then analyzed using the image processing and analysis software (ImageJ version 1.50i, USA) to determine the average Feret diameter and the shape of the beads. The shape of hydrogel bead was determined using a dimensionless shape indicator known as shape distortion factor (SDF), as given in Eq. (1),

$$SDF = (d_{max} - d_{min})/(d_{max} + d_{min}) \quad (1)$$

where d_{max} and d_{min} represent the maximum and minimum Feret diameter, respectively. In general, the SDF value varies from zero to unity for a perfect sphere to a greatly distorted object. A hydrogel bead is considered spherical if the SDF value is < 0.05 [6].

2.2.4.2. Fourier transform infrared (FTIR) spectroscopy. Firstly, the hydrogel beads were lyophilized using a freeze dryer (Alpha 1–2