Accepted Manuscript

Optimization of pH-responsive carboxymethylated iota-carrageenan/chitosan nanoparticles for oral insulin delivery using response surface methodology

Pratyusa Sahoo, Kok Hoong Leong, Shaik Nyamathulla, Yoshinori Onuki, Kozo Takayama, Lip Yong Chung

PII: S1381-5148(17)30160-8
DOI: doi: 10.1016/j.reactfunctpolym.2017.08.014
Reference: REACT 3900
To appear in: Reactive and Functional Polymers
Received date: 31 March 2017
Revised date: 17 August 2017
Accepted date: 30 August 2017

Please cite this article as: Pratyusa Sahoo, Kok Hoong Leong, Shaik Nyamathulla, Yoshinori Onuki, Kozo Takayama, Lip Yong Chung, Optimization of pH-responsive carboxymethylated iota-carrageenan/chitosan nanoparticles for oral insulin delivery using response surface methodology. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. React(2017), doi: 10.1016/j.reactfunctpolym.2017.08.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Optimization of pH-responsive carboxymethylated iota-carrageenan/chitosan nanoparticles for oral insulin delivery using response surface methodology

Pratyusa Sahoo\textsuperscript{a}, Kok Hoong Leong\textsuperscript{a*}, Shaïk Nyamathulla\textsuperscript{a}, Yoshinori Onuki\textsuperscript{b}, Kozo Takayama\textsuperscript{c}, Lip Yong Chung\textsuperscript{a,*}

\textsuperscript{a}Department of Pharmacy, Faculty of Medicine, University of Malaya, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
\textsuperscript{b}Department of Pharmaceutical Technology, Graduate School of Medical and Pharmaceutical Science, University of Toyama, Sugitani 2630, Toyama-shi, Toyama, 930-0194, Japan
\textsuperscript{c}Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

* Corresponding author. Tel.: +60 3 79674971; fax: +60 3 79674964
E-mail addresses: chungly@um.edu.my, chungly@hotmail.com (L.Y. Chung), leongkh@um.edu.my (K.H. Leong).

Abstract

In designing an oral delivery method for insulin, we previously reported that pH-responsive carboxymethylated kappa-carrageenan-based microparticles protected insulin from acid degradation during transport through the gastrointestinal tract. However, the low surface-to-volume ratio of these microparticles and the presence of only one sulfate group in each kappa-carrageenan subunit for insulin stabilization may lead to a suboptimal delivery efficiency. To improve the delivery efficiency, we designed a nanoparticle from chitosan (CS) and carboxymethylated iota-carrageenan (CMCi) that possessed two sulfate groups per subunit based on response surface methodology together with multivariate spline interpolation (RSM\textsuperscript{MSI}). The resulting optimized nanoparticles had a zeta potential, mean particle size, loading capacity, and entrapment efficiency of $52.5 \pm 0.5$ mV, $613 \pm 41$ nm, $10.7 \pm 0.6\%$, and $86.9 \pm 2.6\%$, respectively. The release of insulin from the optimized nanoparticles was low ($4.91 \pm 0.24\%$) in simulated gastric fluid (SGF) and high ($86.64 \pm 2.2\%$) in simulated intestinal fluid (SIF) during a 12-h release study, thereby showing a pH-responsive drug release property. The nanoparticles were stable at 4°C and −20°C for at
least 90 days and for up to 7 days at room temperature. The RSM^MSI technique successfully expedited the design of the nanoparticles, which could serve as an improved oral insulin drug delivery system.

**Keywords:**
Nanoparticle
Insulin
Chitosan
Carrageenan
Response surface methodology (RMS)
1. Introduction

In recent decades, research on insulin for the management of diabetes mellitus and its mode of administration has captured great interest. Despite being a common route of administration, subcutaneous routes have a number of disadvantages, such as local pain, itching, hypersensitivity, increased insulin levels and insulin lipodystrophy surrounding the injection site [1]. Other routes, such as oral, buccal and pulmonary routes, have been suggested. Oral administration is more convenient and comfortable than the other methods, and it overcomes the above problems. Moreover, oral administration is physiologically more relevant, as insulin is directly delivered to the gastrointestinal tract (GIT) and reaches the liver through hepatic portal circulation, which is the primary site of action, thus producing a similar effect to that of pancreas-secreted insulin [1–2]. However, the proteolysis of insulin in the acidic gastrointestinal tract (GIT) and the poor permeability via the intestinal membrane and the mucin barrier limit its oral absorption. The entrapment of insulin in micro- and nanoparticle carrier systems safeguards the insulin from the harmful gastric environment, preventing acidic and enzymatic degradation [3–4]. Furthermore, mucoadhesive carrier systems extend the insulin residence time in the GIT, which can confine the insulin concentration to the intestinal wall and thereby amplify the bioavailability [5–8].

We have previously reported that carboxymethylated kappa-carrageenan microparticles with a diameter of 1273 µm were capable of protecting their entrapped insulin from degradation in the GIT and provided intestine-targeted insulin release. In rats, the insulin-containing lectin-functionalized microparticles conferred an overall bioavailability of 12.8–14.8% compared to the subcutaneous route [9]. In this study, we sought to improve the delivery of insulin by entrapping it in chitosan-complexed carboxymethylated iota-carrageenan (CS/CMCi) nanoparticles. The advantage of these nano-sized particles is that they possess a higher surface-to-volume ratio than previously reported microparticles [10]. The attachment of a pH-responsive carboxylic acid group to the iota-carrageenan molecule imparts a site-specific swelling and drug release property on the native iota-carrageenan molecule [9–11]. The additional sulfate groups in the carboxymethylated iota-carrageenan (CMCi) molecule also increase its interaction with the amino groups of the insulin molecules and thereby improve the insulin stability and entrapment [9]. CS is incorporated to serve as a complexation agent and to impart mucoadhesive properties to the nanoparticles. Moreover, CS facilitates the transient opening of the tight gap junctions between the epithelial cells of the intestinal mucosa to assist in the transport of the macromolecules across the intestinal
barrier into systemic circulation [12]. Recently, a study reported that the polyelectrolyte complexation of two natural polymers, chitosan and carrageenan, yielded nanoparticles with diameters of 400–600 nm [13]. This polyelectrolyte complexation method for nanoparticle preparation uses very mild procedures, which avoids deleterious organic solvents or elevated mechanical forces. Hence, this complexation method is safe for protein drug entrapment.

The process of optimizing the carboxymethylation of iota-carrageenan and formulating CS/CMCi nanoparticles with the optimal insulin entrapment efficiency and release profile for oral administration is tedious and time consuming. This is because of the numerous variables involved, which include the degree of carboxymethylation of iota-carrageenan, the ratio of chitosan to carrageenan, the swelling and gelling properties of the nanoparticles, and the insulin release profile in different pH environments. Among the various computational methods, a response surface methodology together with multivariate spline interpolation (RSM\textsuperscript{MSI}) has a compound correlation between variables and responses, giving a firm and consistent optimal solution [14–18]. Therefore, we adopted an RSM\textsuperscript{MSI} approach to optimize the process and develop the ideal formula for the preparation of suitable insulin-entrapped CS/CMCi nanoparticles for oral delivery of insulin. The experimental flow chart of this study is depicted in Scheme 1.

Thus, in the present study, insulin-loaded carboxymethylated iota-carrageenan/chitosan nanoparticles were formulated by the polyelectrolyte complexation method. The carboxymethylation of iota-carrageenan and nanoparticle formulation were optimized by RSM\textsuperscript{MSI} to determine the appropriate reaction conditions for the optimized formulation. The nanoparticles were further characterized by their particle size, zeta potential, surface morphology, entrapment efficiency, drug loading, in vitro insulin release in both simulated gastric fluid (SGF) (pH 1.2) and simulated intestinal fluid (SIF) (pH 6.8) and the storage stability of the entrapped insulin.

2. Materials and methods

2.1. Materials

*Iota*-carrageenan (batch no.: 405301) was acquired from Marine Science Co., Ltd. (Tokyo, Japan). NaOH, monochloroacetic acid, potassium dihydrogen phosphate, sodium chloride, hydrochloric acid, ortho-phosphoric acid, acetic acid, dipotassium hydrogen phosphate, acetonitrile, sodium sulfate, and deuterium oxide were purchased from Merck
(Darmstadt, Germany). 2-Propanol and ethanol were obtained from Fisher Scientific UK Ltd. (Loughborough, Leicestershire, UK). Chitosan (low molecular weight, 50–190 kDa; deacetylation ≥ 75.0%; batch no.: SLBG5615V) and human recombinant insulin (≥ 27.5 IU/mg) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation and characterization of CMCi

2.2.1. Experimental design

A Box-Behnken design (BBD) with four factors and three levels was used to optimize the carboxymethylation of iota-carrageenan. The four selected independent factors included the volume of NaOH (X1), the concentration of NaOH (X2), the amount of ClCH2CO2H (X3) and the reaction temperature (X4), which generated 33 different experimental runs listed as I01 to I33 (Table S1). The dependent variables included the degree of swelling of insulin-free CS/CMCi nanoparticles in SGF (pH 1.2) (Y1), the gel fraction of insulin free CS/CMCi nanoparticles in SGF (pH 1.2) (Y2) and the release of entrapped insulin (Korsmeyer-Peppas model (Eq. 1) parameters k (Y3) and n (Y4) for the CS/CMCi nanoparticles in SGF (pH 1.2) [19–20]. The same parameters were investigated for the nanoparticles in SIF (pH 6.8), including Z1: the degree of swelling of insulin-free CS/CMCi nanoparticles; Z2: the gel fraction of insulin-free CS/CMCi nanoparticles; Z3: parameter k and Z4: parameter n. SGF and SIF were prepared according to the British Pharmacopeia (BP 2013). Both the independent factors and the dependent variables were simultaneously optimized to obtain the optimal formula.

\[
\frac{M_t}{M_\infty} = k t^n \quad \text{(Eq. 1)}
\]

In this equation, \( M_t/M_\infty \) is the fraction of insulin released at time t, k is the structural/geometric constant for a particular system, and n is the release exponent representing the release mechanism. Statistical data analyses were performed using Student’s paired t-tests, where \( p < 0.05 \) was considered to be statistically significant.

2.2.2. Characterization of CMCi

The CMCi samples were synthesized according to the previously mentioned protocol for the carboxymethylation of kappa-carrageenan [19]. For NMR analyses, the iota-