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Research paper

Optimizing electrostatic interactions for controlling the release of proteins from anionic and cationically modified alginate

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ABSTRACT

Alginate and cationically modified alginate microparticles were prepared with the goal of developing hydrogel microparticles that offer controlled release of protein drugs mainly by modification of the absolute charge of the hydrogel network. Protein loading and release studies were carried out using model proteins with different net charges (i.e. low, high, and neutral isoelectric points) covering a broad range of molecular weights. The Projection to Latent Structures (PLS) method was used for qualitatively and quantitatively describing the relationships between the properties of proteins such as net charge and molecular weight, polymer properties including degree of substitution and microparticle size, and the release kinetics (kt^n). It was found that electrostatic interactions and protein molecular weight had the greatest impact on parameter k while parameter n was mostly affected by polymer and buffer properties. In addition to understanding the current trends, the multivariate statistical method also provided an effective and reliable model as a beneficial tool for predicting and optimizing protein delivery systems.

1. Introduction

Delivery of proteins remains a significant challenge in the field of drug delivery. One promising method for optimizing and controlling protein release from hydrogels involves taking advantage of the electrostatic interactions between the protein and hydrogel [1,2]. The distinct surface composition of proteins imparts a charge and depending on the pH of the solution and the isoelectric point (pI) of the protein, a different net charge may be displayed at its surface [3]. At pH values below the isoelectric point, proteins carry a net positive charge, due to a high degree of protonation of the amine groups and a low degree of dissociation of the carboxyl groups. At pH values higher than the isoelectric point, proteins carry a net negative charge, as a result of the high degree of dissociation of the carboxyl groups and the low degree of protonation of the amine groups [4]. However, it should be noted that the surface charge may be very different than the net overall charge [5].

Electrostatic interactions between hydrogels and proteins must however be optimized in order to control the protein release. Attractive interactions hinder protein release whereas repulsive interactions can increase the rate of release [3]. Charged hydrogels including ionic polymers such as agarose [6], gelatin [7,8], carrageenan [1,9] and alginate [10,11] or hydrogels modified with amino [12], sulfonyl [13] or phosphate [14] functional groups have been used for drug and protein delivery [3] although there are limited studies on the effect of electrostatic interactions between the charged hydrogel networks and proteins on the protein release.

Alginate is an anionic polysaccharide [15] composed of mannuronic (M) and guluronic (G) acid residues [16]. Alginate is a non-toxic and degradable polymer [15] and has been broadly studied for microsphere preparation [17]. Furthermore, in the presence of multivalent cations, alginate ionically crosslinks to form a gel at room temperature and under mild conditions, a process free from the use of organic and toxic solvents [15,17]. Among the different cation-alginate gels, calcium-alginate hydrogels are the most widely used carriers in enzyme, protein and drug delivery applications and are considered to be clinically safe [15,18].

Chemical modifications of alginate have been extensively studied for various applications. For example, sulfated alginate shows great blood compatibility due to its structural similarity to heparin, which is known as an anticoagulant agent [19]. Oxidization of alginate is known to offer control over its *in vivo* degradability [20], an important aspect in controlled drug delivery applications. Amidation of alginate has been carried out for introducing amphiphilic properties to the polymer network [21]. Through amide bond linkages, Tan et al. [22] synthesized aminated alginate grafted with

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thermo-sensitive polymer for assessing its potential in tissue engineering applications. Li et al. [23] have also reported on synthesis of aminated alginate using aqueous carbodiimide chemistry for enzyme immobilization.

While alginate is an anionic polymer, grafting amine groups on its backbone leads to synthesis of positively modified alginate. To the best of our knowledge, the possibility to control the release rate of proteins by modification of the absolute charge and the charge density of the hydrogel network has not been studied thoroughly to date. Understanding the mechanisms and extent of effects involved in drug release is fundamental for design of drug delivery carriers which can fulfill the therapeutic needs. In this work, the Projection to Latent Structures (PLS) method is used as a multivariate statistical analysis method to provide insight into relationships and correlations between the protein and polymer properties and the release kinetics. The reduced dimensional space (the latent variable space) [24] provides a beneficial tool for understanding the trends, predicting future kinetics, and optimizing the delivery carriers for efficient protein deliveries.

In this study the relationship between protein release kinetics from anionic and cationically modified alginate microparticles was studied considering factors such as net charge and molecular weight of the investigated proteins. Using multivariate statistical methods, the work is extended by predicting the release profiles of other proteins. It is hypothesized that an appropriate protein delivery vehicle can be developed by understanding and developing relationships between the release profiles of the studied proteins and their net charge and molecular weight and adapting the vehicle accordingly.

2. Materials and methods

2.1. Materials

Sodium alginate from brown algae was purchased from Sigma-Aldrich (Oakville, ON). The molecular weight of this alginate is in the range of 100,000–200,000 g/mol. This alginate consisted of 65% guluronic acid 35% mannuronic acid residues. 1-(3-Dimethyla minopropyl)-3-ethylcarbodiimide (EDC) was purchased from Carbosynth Limited (Berkshire, UK). 1-hydroxybenzotriazole (HOBt) was obtained from Toronto Research Chemicals (Toronto, ON). N, N-Dimethylethylenediamine (DMEN) was purchased from Sigma-Aldrich (Oakville, ON). All other reagents and proteins (insulin, bovine serum albumin, lysozyme, chymotrypsin, myoglobin, horseradish peroxidase) were also obtained from Sigma-Aldrich (Oakville, ON).

The phosphate buffered saline 1X was composed of NaCl (138 mM), KCl (2.7 mM), NaH_2PO_4 (1.9 mM), and Na_2HPO_4 (8.1 mM). This buffer is also referred to as PBS 10 mM in literature, which is the concentration of the phosphate ions. In this study, the 10-times dilution of the PBS 1X is denoted as PBS 0.1X (or PBS 1 mM).

2.2. Alginate modification

Cationically modified alginate were prepared by grafting amine groups onto the alginate backbone using aqueous carbodiimide chemistry. Alginate was dissolved in deionized water to obtain a 1.5% (w/v) alginate solution. Prescribed amounts of EDC (5 M excess relative to carboxyl groups) and HOBt (1:1 mole:mole EDC) were added to the alginate solution and the pH of the solution was adjusted to 5 using 1 M HCl. The mixture was stirred (500 rpm) at room temperature for 30 min for full activation of the carboxyl groups on the alginate. During the reaction, the pH of the solution was monitored and if necessary, adjusted to 5 using 1 M HCl. Then, various amounts of DMEN (for achieving 20, 40, 60, 80 and 100% theoretical degree of substitution of carboxyl groups) were added to the mixture and the reaction was left to proceed for 12 h under constant stirring (500 rpm) at room temperature. The resulting product was dialyzed against a 1 M NaCl solution in deionized water using a 12,000–14,000 MWCO dialysis tube. The synthesized amine modified alginate was then lyophilized followed by storage in sealed containers at room temperature. Fig. 1 illustrates the mechanism of the reaction.

2.3. Characterization of the modified alginates

The synthesized amine modified alginate polymers (Am-Alg) were characterized by FT-IR and ^{1}H NMR for determination of amine conjugation.

FTIR spectra were collected by applying 64 scans with a resolution of 4 cm^{-1} to lyophilized polymers and data between 370 and 5000 cm^{-1} were recorded (Hyperion 3000 FT-IR Microscope, Bruker Corporation, MA, USA).

For ^{1}H NMR spectroscopy, polymer solutions (10 mg/mL) were prepared in D_2O and their ^{1}H NMR spectra were recorded on the 600 MHz spectrometer (Bruker 600 MHz spectrometer, Bruker Corporation, MA, USA). The degree of substitution (DS) was determined from the areas (I_{CH3}) of the signal at δ 2.96 ppm due to the resonance of the methyl group and of the signals attributed to the proton on carbon-1 of guluronic acid, H_1, (signal at 5.10 ppm) [25,26], based on Eq. (1).

$$DS = (I_{CH3}/6)/(I_{H1}/0.65) \quad (1)$$

In Eq. (1), division by 0.65 is done to account for all monomeric units; since the signal at δ 5.10 ppm corresponds to H_1 of only G blocks, which according to supplier (Sigma-Aldrich) make up for ~65% of the alginate molecule.

2.4. Synthesis of microparticles

Alginate microparticles were prepared by the modified emulsification and external gelation methods [27]. A 1.5% or 3% (w/v) solution of alginate in deionized water was prepared and dispersed into paraffin oil containing 5% Span 80 with a ratio of 1:4. After stirring at 1000 rpm for 30 min, a solution of calcium chloride ($CaCl_2$) (0.1 M or 1 M) was added dropwise to the emulsion mixture. The mixture was continuously stirred for 2 h, followed by the addition of isopropyl alcohol for hardening the formed microparticles. Microparticles were collected by centrifugation, washed with isopropyl alcohol and water, and dried at 37 °C.

Microparticles from amine modified alginate polymers were prepared by a similar emulsification and external gelation method. The amine modified alginate and the $CaCl_2$ solutions used in preparation of the particles had concentrations of 1.5% (w/v) and 1 M, respectively.

2.5. Particle characterization

Physical characterization of resulting particles was carried out by measuring particle size distributions (Mastersizer 2000 equipped with Hydro 2000S, Malvern Instruments Ltd., UK) and investigating the zeta potential of the microparticles (Zeta PALS, Brookhaven Instruments Corp., NY, USA).

The morphological structure of the particles was examined using scanning electron microscopy (SEM, Vega II LSU, TESCAN, Czech) on gold sputtered samples.