Bioencapsulation of protein therapeutics within pH-responsive, semi-synthetic alginate based biomaterials for transmucosal pharmaceutical applications

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Introduction

Natural polymers like alginate are of considerable interest in the fields of biotechnology and biomedical engineering and as pharmaceutical controlled release devices (Degim and Celebi 2007). The benefits in the use of natural polymers are considerable due to the biodegradable and biocompatible nature (Augst, Kong et al. 2006). Alginate, one of the most abundant polysaccharides, is extracted from brown algae, and consists of (1→4)-O-glycosidic links of β-D-mannuronic (M) and α-L-guluronic acid (G) residues in varying sequential arrangements and proportions. The pH-sensitive alginate based hydrogels are of particular interest in the development of transmucosal pharmaceutical formulations for the delivery of therapeutic proteins. In acid environment, alginate gel contracts due to limited solubility of non-ionized acid moieties and protects protein from enzymatic and acid degradation in the gastric environment of the stomach (~ pH 1.2) while swelling in alkaline media as a result of increased hydrophilicity of ionized carboxylate moieties, releasing active therapeutics in the intestinal lumen (~ pH 7.8).

To enable the control of properties generally not possible with the native polymer, we have chemically modified alginate with di-aldehyde via acid-catalyzed acetalization. The kinetics of acetalization measured through equilibrium swelling of the networked polymer, were found to undergo zero and second-order reaction with respect to dialdehyde and alginate respectively(Chan, A. et al. 2008). With the determined rate constants of 19.06 $\mu L \cdot mole^{-1} \cdot s^{-1}$ at 40°C and activation energy of 78.58 $kJ \cdot mol^{-1}$, a proposed predictive reaction model may be used *a priori* to select reaction conditions providing specific polymer properties(Chan, A. et al. 2008). Gel swelling and average pore size were then able to be controlled between 80-1000 fold and 35-840 nm respectively, by predictive estimation of reagent concentration and formulation conditions[3]. As a proof of concept, we used this superabsorbent for encapsulation and controlled release of biologicals as potential oral drug delivery vehicles.

Materials and Methods

Preparation of networked alginate by two-step emulsification gelation method

Alginate based pH-responsive superabsorbent was synthesized by a two-step semi-batch condensation reaction whereby preformed acid gel beads were first precipitated from water-in-oil emulsion followed by crosslinking linear polymer with glutaraldehyde in aqueous medium, forming acetal linked three-dimensional gel network. Alginate sol of 4% (SF120, F_G =0.69 and 308 kDa (FMC biopolymer, Brokeroya, Norway) was emulsified in canola oil at a ratio of 1/4 (v/v of alginate sol to oil) by mechanical mixer at 600 rpm for 15 min. Sorbitan monooleate (Span 80) of 0.05% v/v was added to reduce the size bead size distribution. The gelation step involved pH reduction of the sol from ~6.5 down to ~2, resulting in self-assemble acid gel bead. Hydrochloric acid was added at 0.01 (v/v acid to emulsion) to the emulsion, followed by the addition of pre-dissolved glacial acetic acid in oil at 0.05 v/v to initiate gelation of the emulsified alginate droplets.

Mixing was continued for an additional 30 min. pH 1.2 HCl solution was added to the slurry at 1:1 volume ratio to facilitate gel microsphere partitioning into the aqueous phase. The oil supernatant was withdrawn and discarded. Glutaraldehyde solution (25% w/w EM grade packaged in 10 mL single dose ampoule and sealed under dry nitrogen, Sigma-Aldrich, Oakville, Canada), and HCl were introduced to the aqueous medium. The chemical reaction consisted of 2.54 M of chemical crosslinker and 0.42 M of acid catalyst was carried out at 40^{o}C, unless otherwise specified. Chemical gel beads were removed from the reaction medium at specific time interval for the subsequent kinetic study whereby beads were dialysed against of 0.1 M, pH 7.8 NaCl solution for 72 hours and the swollen bead diameters were measured microscopically using a Leica stereomicroscope (D3, Germany).

Absorptive protein encapsulation within chemically network alginate

Equilibrium swollen network alginate gel beads at pH 7.8 were subsequently loaded with model proteins including vitamin B12, insulin, subtilisin, bovine serum albumin, and urease by immersing preformed chemical beads of 2.0 +/- 0.1 mm in protein solutions. Gentle mixing at 150 rpm was provided throughout the uptake until the absorption isotherm reached equilibrium. The resultant active beads were dried by solvent extractive drying whereby microspheres were rinsed with cold acetone at 4^{o}C, followed by immersed in acetone for 15 min to extract residual water. Residual acetone was allowed to evaporate resulting in dry granules.

Results and Discussion

Effect of alginate monomeric composition and molecular weight on the gel formation

The effect of polymer molecular weight and monomeric composition in alginic acid gel on the reaction kinetics was studied and the crosslinking kinetic profiles are plotted in figure 1. The kinetic profiles of acid gel of similar molecular weights (curves B and C, and curves D, E and A-1 of Figure 5) but different in monomeric compositions were similar, indicating no apparent effect of composition and sequential arrangement of the two hexuronic acid monomers on the acetalization reaction. As compared to physical gels in which gel properties depend primarily on the G-monomers due to the spatial arrangement of the ring and hydroxyl oxygen atoms, the formation of crosslinked gel exhibits no apparent dependence on the fraction of G- or M-acid residues. Therefore, it appears that reaction kinetics are molecular weight dependent with very limited effect from the stereochemistry of the two hexuronic acid monomers. This similar reactivity between the two hexuronic acid epimers may allow some alginate types which do not form stable ionotropic gels to be used in the formation of chemically network hydrogel, extending the spectrum of the gelling properties of alginates.

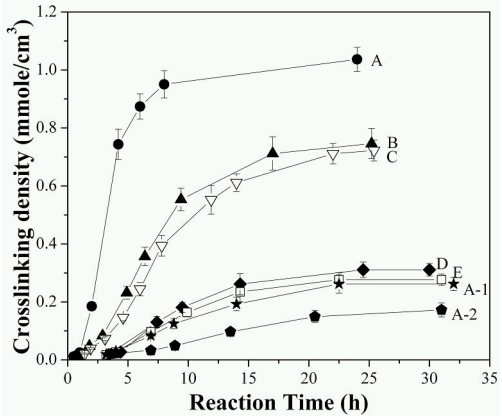

Figure 1. Influence of monomeric compositions, and primary molecular weight of alginic acid on crosslinking kinetics. The molecular weights of the samples studied were characterized by the intrinsic viscosity with the descending order: 8.63 (A,●), 7.89 (B, ▲), 7.48 (C,▽), 4.95 (D,◆), 4.70 (E, □), 4.45 (A-1, ★), and 2.22 dL/g (A-2, ⬟). Samples A,B, D, A-1 and A-2 consisted of high G-content (closed symbols) whereas samples C and E (open symbols) were medium G-content alginate.

Oscillatory pH-responsive swelling properties

Oscillatory swelling behaviour of alginate chemical gel beads was examined to characterize the reversibility/repeatability of the swelling process. Fresh alginate chemical gel beads were submerging in NaCl solution with solution pH alternating between 7.8 and 1.2 every 60 min. Dynamic swelling behaviour is presented in figure 2. The network polymer experienced pronounced and repeatable swelling characteristics when alternating between acidic and alkaline pH. The magnitude of swelling is depended on the network structure and the external pH and ionic environment. Through carefully controlling the reaction kinetics, the crosslinking density can be manipulated, ultimately the water holding capacity, matrix pore size, and mechanical properties of the network can be controlled.

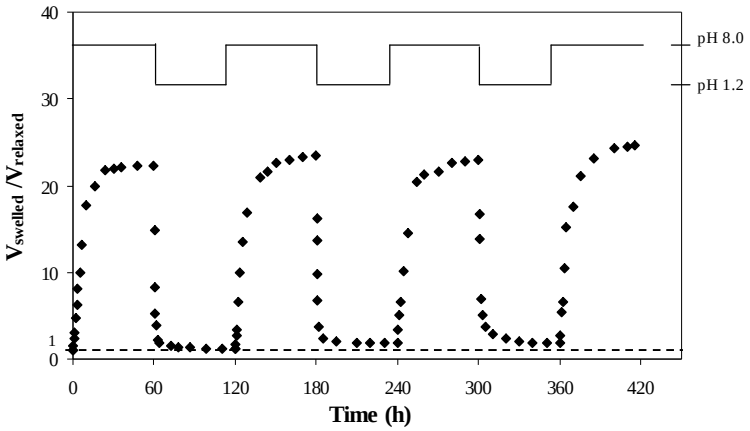

Figure 2. The oscillatory swelling response of networked alginate hydrogel to repeated pH-stimulus. The swelling ratio was the volume at the swelled state to the relaxed state defined as the gel beads formed after the chemical crosslinking but before the swelling test.

Characterization of semi-synthetic alginate network polymer in simulated gastric-intestinal conditions

The pH-dependent swelling behaviour of semi-synthetic alginate polymer was characterized by atomic force microscopy. In acid environment, alginate gel contracts due to limited solubility of non-ionized acid moieties while swelling in alkaline environment as a result of increased hydrophilicity of ionized carboxylate moieties. As can be seen in Figure 3, the pore structure of the polymer remained compacted at low pH and increased from less than few nanometers to approximately 100-200 nm when placed in alkaline solution, and so is under consideration as a potential oral drug delivery vehicle.

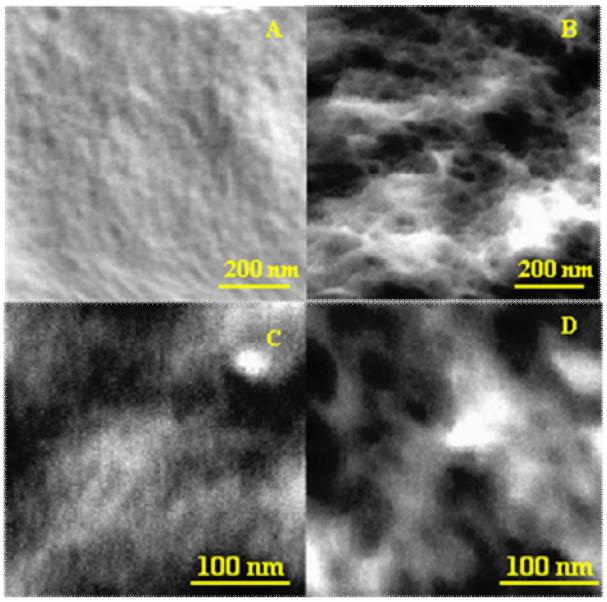

Figure 3. Tapping-mode AFM topographs of alginate granules swelled in pH 1.2 (A) and in pH 7.8 (B). (C) and (D) are images of higher magnification of a section of image A and B, respectively.

Encapsulation of wide range of active biologicals within pH-responsive superabsorbent

A technology platform which decouples bead production from the subsequent encapsulation process is proposed, offering cost effective large scale bead production, providing customized bead properties, and high encapsulation yield(Chan, Becker et al. 2005). As a proof of concept, model proteins of wide range molecular weights including vitamin B12, insulin, subtilisin, BSA, and urease were absorptively encapsulated within semi-synthetic alginate polymer. Protein release profiles in simulated gastric intestinal tract are presented in Figure 4. Although, approximately 15 to 65% of cumulative protein release was experienced during the transient period (0-60 min), it is apparent that proteins retained within alginate granules beyond the transient period (>60 min), except for vitamin B12 at low pH. At alkaline condition (ie. intestinal pH), granules began swelling, facilitating protein release. The semi-synthetic alginate demonstrated trigger-release properties in the simulated GI environments, a potential oral drug delivery vehicle for protein therapeutics.

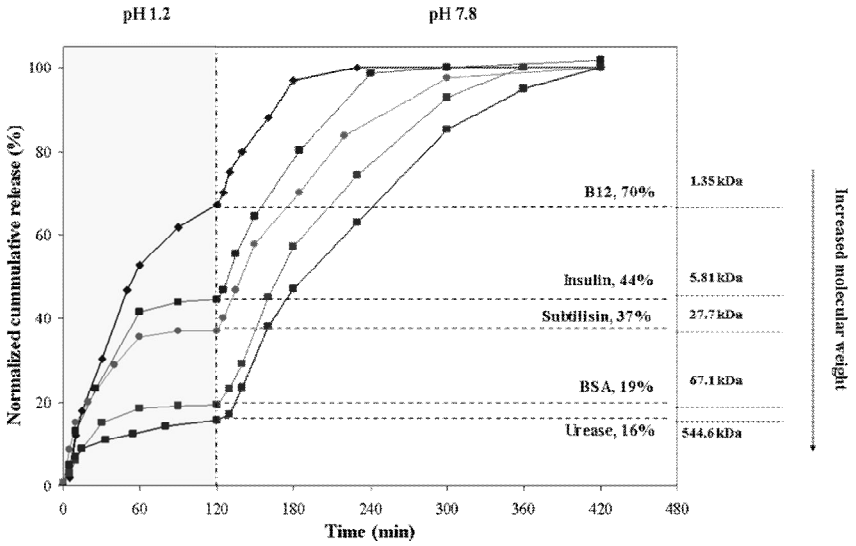

Figure 4. Release profiles of various biomacromolecules in simulated GI environment.

Conclusion

Semi-synthetic network alginate exhibiting pH-sensitive swelling characteristic was synthesized by a Lewis acid catalyzed acetalization reaction between alcohols and aldehydes. The two C-5 epimers in alginate demonstrated similar reactivity, but increased alginate molecular weight, acid catalyst concentration and reaction temperature, increased the rate of acetal formation. A proposed predictive reaction model may be used *a priori* to select reaction conditions providing specific polymer properties. As a proof of concept, a wide range of molecular sizes of biomacromolecules were absorptively encapsulated into alginate based biomaterial. The resultant granules containing bioactives demonstrated triggered release properties in the simulated GI environments.

References

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