Alginate micro- and nanoparticles are produced by spray-drying for oral delivery of therapeutic peptides and protein

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Introduction

Currently, therapeutic proteins and peptides are delivered subcutaneously, as they are readily denatured in the acidic, protease rich environment of the stomach or gastrointestinal track and have low bioavailability results from poor intestinal absorption through the paracellular route. Encapsulation of therapeutic peptides and proteins into polymeric micro- and nano- particle systems has been proposed as a possible strategy to overcome limitations to oral protein administration (Reis *et al*, 2006). Furthermore, it was shown that nanoparticles having diameters less than 5µm are able to be taken up by the M cells of Peyer's patches found in intestinal mucosa (Hussein *et al*., 2001).

Several formulation techniques have been investigated previously to produce nano particles (<5µm), including nanoemulsion dispersion (Reis *et al.*, 2004) and ionotropic pre-gelation (Sarmento *et al*, 2006). Although, the desired particle sizes were achieved these methods have the drawback of requiring organic solvents and multiple steps. However, spray drying is a single step process, which can be operated continuously. In this study, a single encapsulation step procedure involving nanospray drying was used to prepare nanoparticles with potential for oral administration of therapeutic proteins. The method involved introduction of dilute Ca^{++} to a dilute alginate feed solution, along with the protein and glycol-chitosan, where ionic crosslinking of the alginate and polyion complex formation takes place forming sprayable low viscosity gel. The resulting particles were investigated for size, protein content and protein release kinetics. The distribution of the model protein throughout the polymer matrix was examined by using confocal laser scanning microscopy with florescent labeled protein.

Materials and Methods

Materials:

Low viscosity sodium alginate (Sigma-Aldrich, Oakville, Canada) with specifications: 250 cP for 2% solution at 25°C; molecular weight about 147,000; 61% mannuronic acid; batch number 112K0931.

Methods:

Preparation of Micro/Nano Particles

Sodium alginate was dissolved in deionized water, then dearated for 30 min. Subtilsin, BSA, or lysozyme as model proteins were dissolved at the noted concentrations, into the alginate solution. The alginate-protein solution (100 mL, 1.5%, w/v) at an alginate/protein ratio of 9:1, were prepared. The $CaCl_2$ solution (350 mL, 10 mM) was added and dispersed by mixing for 5 minutes. Glycol chitosan solution was prepared in distilled water (50 mL, 0.8%), then mixed with the alginate and dilute Ca^{++} solution. The feed solution containing alginate, dilute Ca^{++} and glycol-chitosan, carrying the desired protein was spray dried using a Buchi-290 Mini Spray Dryer (Buchi Laboratories, Technik AG, Flawil, Switzerland). During spray drying, the feed solution was stirred continuously. The dryer nozzle and glass walls of the particle collection vessel were cooled with tap water during the drying operation.

Protein release in GI simulated media

Protein release was carried out under simulated gastrointestinal (GI) conditions by suspending 10 mg particles into 20 mL 0.1M hydrochloric acid solution at pH 1.2, 37°C for 2h, followed by transfer to 0.05M phosphate buffer at pH 6.8 for 3h. Experiments were performed in triplicate with mixing. At appropriate time intervals, 1.2 mL aliquots were removed, centrifuged and supernatant removed to be assayed for protein content spectrophotometrically (Cary 1, Varian, Australia) at 595 nm using the Bradford modified method (Coomassie plus kit, Pierce, Fisher, Canada). In order to keep the volume constant, 1.2 mL of the buffer solution was replaced. The percentage of released protein and encapsulation efficiency were calculated assuming that all the protein was released after the first 4 hours from the point when the particles were initially added to pH 1.2. The protein encapsulation efficiency of the particles was determined by the ratio of the initial protein load to the particle formulation to the spectrometrically determined amount of the proteins after rehydration.

Determination of the size distribution of the particles

Spray dried particles were sized using a laser diffraction particle sizer (Malvern Mastersizer 2000). Around 100 mg of particles were analyzed for each batch. For each batch of particles the mean diameters were calculated in triplicate. The size distribution was estimated by a SPAN factor, which is defined by the ratio; $SPAN = (D_{90}-D_{10})/D_{50}$. where $D_{90\%}$, $D_{50\%}$ and $D_{10\%}$, are the mean diameters at which cumulative volume percent of 90, 50 and 10% of the particles are determined. A high SPAN indicates a wide size distribution, whereas a low value indicates a narrow size distribution.

Particle morphology and protein distribution

Morphology was examined by scanning electron microscopy (JEOL, JSM-840) with gold coated particles. Protein distribution within the particle matrix was determined by confocal laser scanning microscope (Leica TCS SP2, Germany). A 3 mL amount of BSA-FITC solution (10 mg/mL) was added to a sodium alginate, Ca^{++}, chitosan solution (alginate/protein ratio of 9:1, for 0.3% Na-A solution), and following spray drying, the particles were analyzed by "pro plus basic" Leica operating software. The imaging was performed with dry particles in order to prevent swelling and release of the protein.

Results and Discussion

Properties of the chitosan-ca-alginate particles

Chitosan-alginate particles were tested for particle size, protein loading and morphology. The final protein loading within the particles and the particle size distribution is presented in Table 1.

Protein	D[0.1]	D[0.5]	D[0.9]	SPAN	Encapsulation [%]
BSA	2.16	3.17	12.8	3.36	96±2
Subtilisin	2.11	3.53	12.1	2.83	94±1
Lysozyme	2.01	3.16	13.1	3.51	95±2

Table 1: Comparison of particle size distribution of chitosan-alginate particles carrying different proteins. The chitosan:protein:-alginate ratio was 4:2:14. The feed solution contained 0.3% alginate and 10 mM Ca^{++}. Spray drying operating conditions; liquid feed rate = 5 mL/min, aspirator Rate = 38 m^3/h , drying air flow rate = 600 L/h, atomization pressure = 80 psi.

The mean particle size D[0.5] was approximately 3 µm. SPAN values showed a relatively broad size distribution. For all of the particles, encapsulation yield was more than 94%.

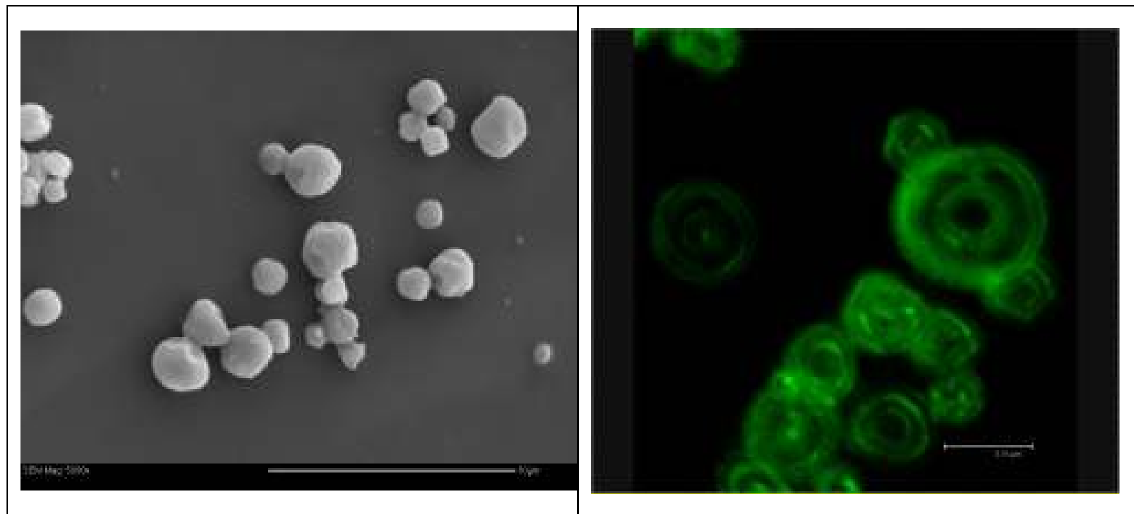

Figure 1: a. SEM images of BSA loaded particles. The scale bar represents 10 µm. b. Confocal laser scanning microscope image of particles carrying BSA. The green sections represent the presence of FITC labeled BSA. Scale bar represents 3 µm.

The protein loaded particles showed spherical morphology with smooth surface as illustrated in Figure 1a. The distribution of the protein throughout the polymer matrix was examined by confocal laser scanning microscopy with florescent labeled BSA, as shown in Figure 1b. The particles show a donut-like structure with hollow center, and protein deposition toward the particle surface. This might be due to evaporation of the water from the droplet surface, which might be carrying the solutes from the center to the surface during formation of the particles.

The results show that chitosan-alginate particles, sizes ranging from several hundred nanometers to a few microns in diameter can be produced by spray drying in a single step operation. The produced particles also had a mean size lower than the critical diameter necessary to be orally absorbed by M cell's of the Peyer's patches in the gastrointestinal tract.

Protein Release

Protein release through a simulated gastrointestinal tract were carried out with chitosan-Ca-alginate particles formulated with low molecular weight model proteins; BSA, subtilisin and lysozyme. Release profile of the particles are presented in Figure 2.

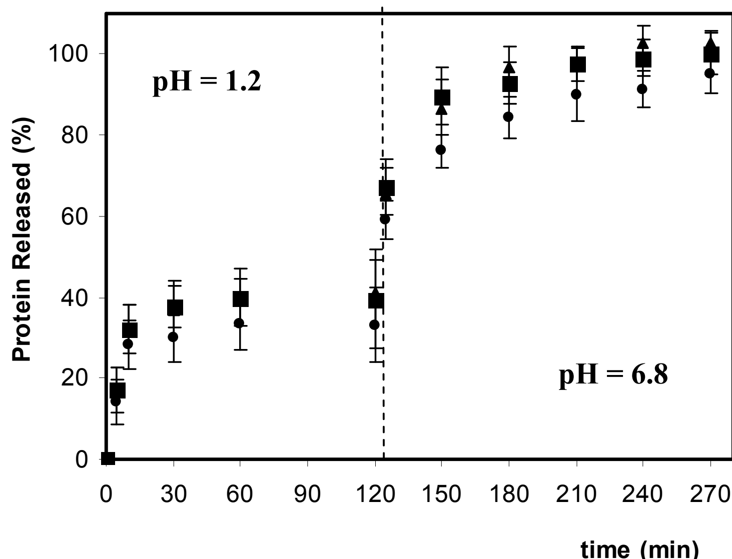

Figure 2: Release profile of BSA (●), subtilisin (▲) and lysozyme (■) from chitosan-alginate particles at pH 1.2 followed by pH 6.8. Simulation at pH 1.2 represents residence time of particles in stomach, and pH 6.8 represents conditions in intestinal tract.

Particles released more than 35% of the protein load within 30 min in the simulated stomach environment. After 2h, the particles were transferred to a simulated intestinal environment at pH 6.8. Within the first 5 min, a second burst release profile was observed, possibly due to the swelling of the particles. Although the positively charged lysozyme (pI = 11.4) and subtilisin (pI = 9.4) had different molecular weights, they showed similar release profiles, possibly due to the large pore size of the resulting particles compared to the molecular size of the particles. However, BSA showed a slightly slower release profile, which might be due to the larger molecular size.

Conclusion

Chitosan-alginate particles were produced in single step operation with spray drying. Particle size ranged from several hundred nanometers to a few microns in diameter. The resulting spherical nanoparticles had an encapsulation yield of more than 94%. The produced particles also had a mean size lower than the critical diameter necessary to be orally absorbed by M cell's of the Peyer's patches in the gastrointestinal tract and thus can be considered as a promising technology for oral peptide and protein delivery.

References

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