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Scientific Research

Preparation and Release Properties of Sol-Gel Encapsulated Proteins

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ABSTRACT

A glycoprotein, bovine serum albumin (BSA) was used as a model compound for encapsulation in a sol-gel matrix. Dried gels were ground into powders and meshed to achieve particle sizes less than 250 μm. The products were washed with phosphate buffer. Capillary electrophoresis was used to evaluate the encapsulation efficiency and the kinetic properties of protein release. Several parameters, including the pH and composition of the background electrolyte, were investigated in an effort to eliminate the matrix effect from the determination of release kinetics. Complete separation of the silica matrix from BSA was using phosphate buffer, an applied voltage of 15 kV, and detection at 278 nm. Kinetic studies indicated that most of the BSA was released in the first 5 h. The rate of BSA release gradually decreased, and some BSA after 25 h. These results indicated that dilute potassium phosphate buffer could accelerate protein release, but this was not observed for the concentrations greater than 50 mM. We believe the developed method has potential utility in other systems for *in vitro* matrix dissolution and drug release studies.

Keywords: Bovine Serum Albumin; Capillary Electrophoresis; Encapsulation; Release; Sol-Gel

1. Introduction

Therapeutic proteins are becoming available for the treatment of a wide range of diseases. Major limitations to the efficiency of protein therapeutics are reduced stability and short circulation half-lives after parenteral administration [1]. As a result of the invasive nature of these routes of administration, injectable formulations are frequently met with patient discomfort and noncompliance. Additional difficulties for proteins *in vivo* include susceptibility to proteolysis and colloidal instability. These factors necessitate either high drug concentrations or high dosing frequencies, which may lead to adverse side effects. There is an urgent need for protein delivery systems that will enable targeted, controlled release profiles. Carriers such as liposomes, polymer micelles, and hydrogels have been developed to achieve protein delivery [2].

There has been considerable interest in the use of sol-gel materials for biological and biomedical applications [3,4]. Operating at the interface of biology, chemistry, and materials science, this process has been used to immobilize biomolecules via entrapment within sol-gel derived matrices. The interpenetrating networks of silica effectively serve to "cage" the biomolecules, and it remains sufficiently loose to allow for local rotational and translational motions, including those required for substrate binding [5]. Bioencapsulation retains not only the structural integrity of the entrapped biomolecules but also, more importantly, their full biological function [6, 7].

Hydrogels are excellent protein containers [8,9]. However, silica gel encapsulation of living cells and other biological components is not yet understood, due to the complexity of sol-gel chemistry and the variety of materials that may be considered for encapsulation [10].

BSA is a well-studied biomolecule among the wide variety of biological species that have been encapsulated in sol-gel matrices [11-13]. Gao *et al*. [14] have studied the structural evolution of sol-gel matrices in the presence of BSA. BSA has been encapsulated in a silica matrix to evaluate its utility for the separation of some drug

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enantiomers [15,16]. A new type of sol-gel/organic hybrid composite material, using gelatin or chitosan with tetramethyl orthosilicate, was developed to prepare a monolithic column for capillary electrochromatagraphic separation of enantiomers of tryptophan [17].

Calcium silicate is also a potent material for biological and biomedical applications. Fujiwara *et al.* [18] reported the preparation of calcium silicate microparticles encapsulating proteins by the interfacial reaction method, and their applications to controlled release and drug delivery. Zhao *et al*. [19] have developed a lower release dual drug delivery system consisting of a calcium phosphate nanocarrier loaded with both BSA and the drug ibuprofen.

Over the last decade, capillary electrophoresis (CE) has achieved as a fast, powerful, efficient, cost effective and high resolution separation technique. Igartua *et al*. [20] investigated the stability of BSA encapsulated in poly (D,L-lactide-co-glycolide) microspheres using CE. Chen *et al*. [21] have used CE to determine the concentrations of free and encapsulated oligonucleotides in a liposomal formulaton of a drug product. Nguyen *et al*. [22,23] reported that they had developed an efficient quantitative CE-ICP-MS method to characterize a liposomal formulation of the anti-cancer agent oxaliplatin. In this work, porous silica particles prepared by the sol-gel-process were investigated as encapsulation matrices for the controlled release of BSA. This protein release was controlled by careful choice of buffer pH and buffer concentration. The retention performance of the released BSA was studied by CE. The release kinetic of the encapsulated protein was also subsequently determined.

2. Experimental

2.1. Instrumentation

All experiments were performed using a laboratory-built unit. The unit consists of a ± 30 kV high voltage power supply (EH 30P3, Glassman, USA) and a UV-Vis detector (870-CE, Jasco, Japan). Electropherograms were recorded and processed with a CT-21 data processor (Peak-ABC, Singapore). The fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) had a 75-μm i.d. and a 375-μm o.d. with a total length of 75 cm and a distance of 55 cm between the injection point and the detection window.

2.2. Reagents and Chemicals

BSA, disodium 2,2’-biquinoline-4,4’-dicarboxylate (Na_2BCA) and tetraethyl orthosilicate (TEOS) were obtained from Sigma (St. Louis, MO, USA). Cupric sulfate, sodium carbonate and sodium tartrate were obtained from Acros (Geel, Belgium). Purified water (18 MΩ-cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions.

2.3. Immobilization of BSA

TEOS sols were prepared by sonicating a mixture of TEOS (4.5 mL), pure H_2O (1.4 mL) and 0.1 M HCl (100 μL) for 1 h at ambient temperature until it was visibly homogeneous. The sols were stable at −20˚C for as long as two weeks. Xerogels were prepared by mixing TEOS sol (2.4 mL) with 3.9 mL phosphate buffer (50 mM, pH 7) containing 30 μg/mL BSA for protein doped preparations. The mixtures were stored at 4˚C until gelation occurred. The gels were then ground into fine powders, at which point they were ready for use.

2.4. Bicinchoninic Acid (BCA) Microassay [24]

Reagent A was an aqueous solution (100 mL, pH 11.25) of sodium carbonate monohydrate (0.8 g) and sodium tartrate (1.6 g). Reagent B was prepared by dissolving BCA (4 g) in pure water and diluting to the mark (100 mL). Reagent C was cupric sulfate pentahydrate (0.4 g) dissolved in water (10 mL). The assay reagent was prepared to have a volume ratio of reagent A:B:C equal to 26:25:1. Equivalent volume ratios of assay reagent relative to protein standard were prepared and incubated at 60˚C for 1 h. After cooling to the room temperature, the absorbance was measured at 562 nm to construct the calibration curve.

Protein release studies were conducted with various sol-gel particles (30 mg). They were immersed in phosphate buffer (50 mM, pH 7.0). At predetermined time points, the sample was filtered and the supernatant was injected hydrostatically into the CE system. In each experiment, the sample was analyzed in triplicate.

2.5. Capillary Electrophoresis

A fused-silica capillary was flushed prior to use with 0.1 M NaOH, followed by pure water, for at least 30 min each. The capillary was then flushed with 0.1 M HCl for 30 min followed by pure water for an additional 30 min.

Before analysis, the coated columns were preconditioned with the running buffer. Between sample runs, the columns were rinsed with pure water and running buffer for 1 or 2 min intervals. The samples were injected by siphoning at a height difference of 20 cm for 10 s. The samples were monitored at 278 nm with the UV absorbance detector.

3. Results and Discussion

3.1. BCA Microassay

We have adopted the method for protein assays because BCA better tolerates the presence of compounds that interfere with the Lowry assay. It is not affected by various detergents and denaturing agents, such as urea and guanidinium chloride, although it is more sensitive to the

presence of reducing sugars. The calibration curve was prepared over a sample concentration range from 0.4 µg/mL to 20 µg/mL. The formula of the calibration curve as derived from the least square method was $y = 0.0436x + 0.1613$.

3.2. Immobilization of BSA

The sol-gel reaction involves the following steps [25]: 1) hydrolysis of alkoxysilane; 2) condensation of hydrated silica to form siloxane bonds; and 3) polycondensation linkage of additional silanol groups to form cyclic oligomers. Protein molecules are trapped within the silicate network as it forms.

3.3. Release Rate in Different Buffer Compositions

The release buffer was similar to the BGE of CE in each case. The rate of protein release from the sol-gel was determined in buffer solutions at different pH values (pH 5, 7, 8 and 9). Increasing the buffer pH enhances the electrophoretic mobility of the BSA macromolecule. Greater EOF would also occur at higher pH. The net result revealed a faster migration of the BSA macromolecule (**Figure 1**). Interestingly, there are two sample peaks which were assigned to be the isoforms of BSA at phosphate buffer concentration of 20 mM and pH above 5. At buffer pH 9, much greater amount of BSA was released by comparison with those at lower pH (**Figures 1(c)** vs. **(a)** & **(b)**). Meanwhile, a slightly different electropherogram from that shown in **Figure 1(b)** was demonstrated. There are two possible explanations for the phenomena. Either the migration order of the isoforms reversed or the amount of the isoforms altered as the condition changed. Conformational transitions of BSA dependent on pH and ionic strength might be the reason [11,26-28].

Figure 2 shows the release behavior of BSA encapsulated in the sol-gel. Most of the BSA was released in the first 5 h. A slower release rate was then observed, and some BSA remained encapsulated after 25 h. Gao *et al.* [29] have investigated the mechanism by which encapsulated BSA is released from polylactic acid-hyperbranched polyglycerol conjugate nanoparticles. The overall mechanism of BSA release from the nanoparticles is heavily dependent on copolymer relaxation, protein diffusion, protein dissolution or combinations thereof. The cumulative BSA release curves exhibited a burst effect followed by a slowly continuous release phase. Similar release behavior (**Figure 2**) was observed in our own work.

The effect of buffer concentration on the protein release behavior was also investigated. In each case, the buffer concentration was varied over 20, 40, 50, 60, 80 and 100 mM at definite pH value. The sample peak in-

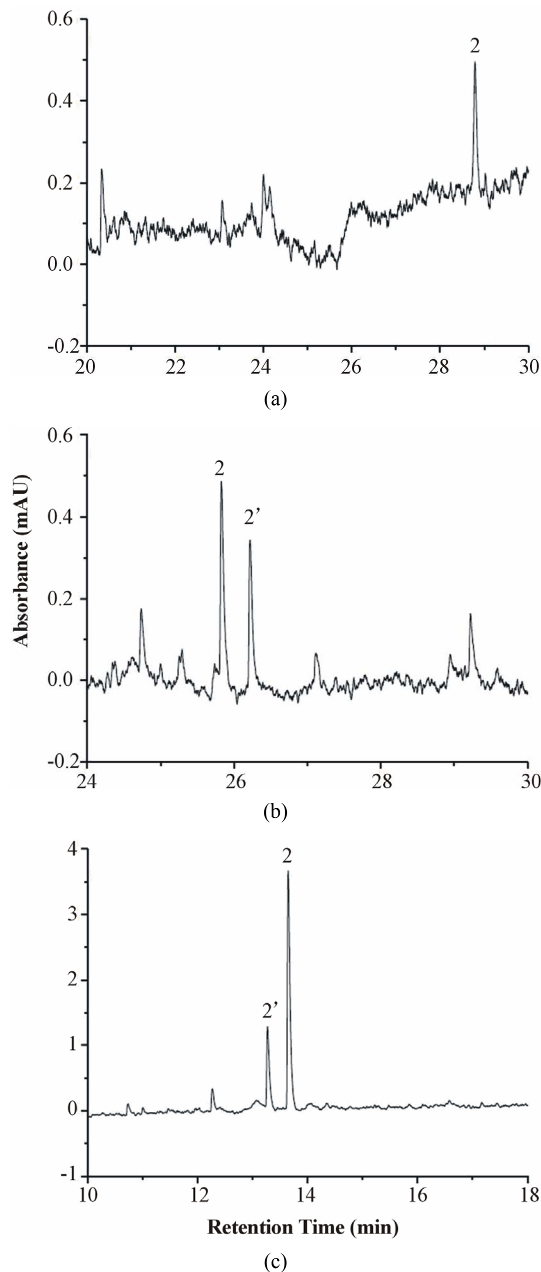

Figure 1. Electropherograms of BSA released from the sol-gel. Column: 75 cm (55 cm) × 75 µm I.D.; sample concentration: 1.13 mg/mL; sample injection: hydrostatic (20 cm, 10 s); applied voltage: 15 kV; detection at 278 nm; phosphate buffer (20 mM) (a) pH 5 (b) pH 7 and (c) pH 9. Peaks: 1. sol-gel matrix (not shown in the scale used). 2 and 2' are isoforms of BSA.

tensity increased significantly as the releasing buffer concentration increased. This relationship was not maintained, however, when the buffer concentration was greater than 50 mM. A summary (3D plot) of the release profile of the encapsulated protein across various buffer compositions is shown in **Figure 3**. The peak corresponding to maximum protein release clearly occurs at

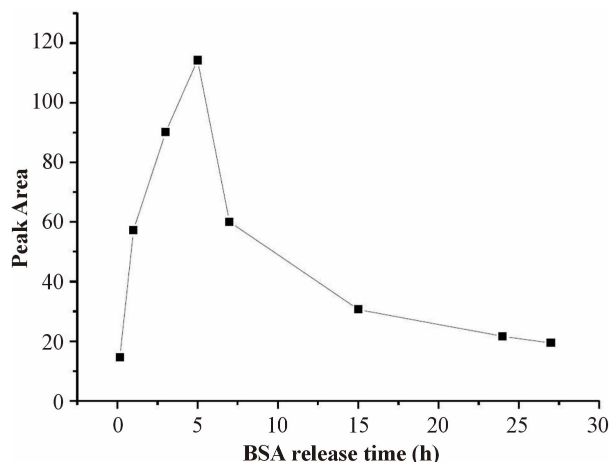

Figure 2. Release rate of the encapsulated protein. Measurement conditions were the same as in Figure 1, except phosphate buffer (50 mM, pH 7).

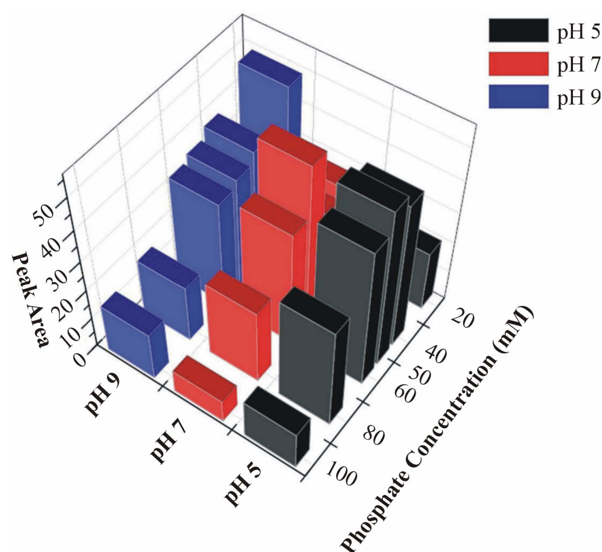

Figure 3. The 3D plot of encapsulated protein released at different compositions of phosphate buffer.

pH 7 with buffer concentrations of approximately 50 mM.

The protein loading capacities of the sol-gel (1.3 mg) were also evaluated. For BSA (1 mL) loadings of 20, 25, 30 and 40 μM, the amount of protein released after 5 h immersed in 1 mL phosphate buffer (50 mM, pH 7) was 54.01%, 45.52%, 39.92% and 34.81%, respectively.

4. Conclusion Remarks

Most of the drug loading and drug release studies were using spectrophotometric analysis including UV-Vis [18, 19,30,31] and fluorimetry [2] or further combined with LC for the matrix isolation. The delivery of biomolecules has also been studied using confocal microscope image [32]. In this work, only BSA was encapsulated in sol-gels. Various surface chemistries can be used for modifying the sol-gel structure to achieve longer delivery periods. Other entrapment materials can also be used to tune this property. Here, we proposed a measurement method for kinetic release studies that is simple, fast and consumes lower reagent quantities. We believe the method that we developed has potential utility in other systems for *in vitro* matrix dissolution and drug release studies.

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