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Designing carboxymethyl cellulose based layer-by-layer capsules as a carrier for protein delivery

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ABSTRACT

Stable hollow microcapsules composed of sodium carboxymethyl cellulose (CMC) and poly (allylamine hydrochloride) (PAH) were produced by layer-by-layer adsorption of polyelectrolytes onto $CaCO_3$ microparticles. Subsequently the core was removed by addition of chelating agents for calcium ions. Zeta potential studies showed charge reversal with deposition of successive polyelectrolyte layers, indicating that the alternate electrostatic adsorption of polyelectrolytes of opposite charge was successfully achieved. The size and surface morphology of the capsules was characterized by various microscopy techniques. The pH responsive loading behavior was elucidated by confocal laser scanning microscopy (CLSM) studies using fluorescence labeled dextran (FITC–dextran) and labeled BSA (FITC–BSA). CLSM images confirmed the open ($pH \leq 6$) and closed state ($pH \geq 7$) of the capsules. A model drug bovine serum albumin (BSA) was spontaneously loaded below its isoelectric point into hollow microcapsules, where BSA is positively charged. The loading of the BSA into the microcapsules was found to be dependent on the feeding concentration and pH of the medium. 65% of the loaded BSA was released over 7 h of which about 34% was released in the first hour. These findings demonstrate that $(CMC/PAH)_2$ hollow capsules can be further exploited as a potential drug delivery system.

1. Introduction

In the rapidly growing field of biotechnology, development of recombinant proteins for therapeutic applications is subject of great interest. However, the physical and chemical instabilities of proteins offer certain challenges in the development and administration of protein therapeutics. The physicochemical and biological properties of proteins such as molecular size, biological half-life, conformational stability, physicochemical stability, solubility and bioavailability [1,2] are different from those of conventional drugs. These intrinsic properties hinder the development and approval of new protein-based therapeutics. Thus, one of the current problems is to design suitable drug delivery systems for these therapeutic molecules.

Many efforts have been devoted toward design and fabrication of nano-structured materials including polymers, hydrogels, organic or inorganic compounds for advanced drug delivery systems. However, fabrication of these materials requires the use of organic solvents which can seriously affect protein stability. To overcome this problem, recently polyelectrolyte microcapsules have been introduced as novel nanoengineered multifunctional materials for drug delivery [3–5].

These capsules have gathered increased interest, as their properties can be easily tailored. Polyelectrolyte microcapsules are obtained by stepwise adsorption of positively and negatively charged polyelectrolytes onto colloidal particles as templates, followed by decomposition of the templates. The stepwise formation allows introduction of multiple functionalities, resulting in unprecedented structure and function. These hollow capsules have a great potential for application in the field of drug delivery [6,7], since their properties and functionalities such as encapsulation and release characteristics can be finely tuned by varying capsule wall thickness, compositions and introduction of exterior stimuli [8]. Polyelectrolyte microcapsules have controllable stability and high permeability for polar molecules [9,10]. As a result, microcapsules have received considerable attention in biotechnology, medicine, catalysis, environment, food, etc. [11–13].

Even though microcapsules can be assembled from synthetic as well as natural polymers, owing to the potential applications of capsules in the fields of biotechnology and drug delivery, considerable interest has been given to the development of microcapsules from natural polysaccharides. Capsules made of natural polysaccharides and derivatives can have significant impact in biomedical field due to their advantages of biocompatibility andbiodegradability.

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Many polysaccharides have been tested for their potential as a drug delivery system. Of these CMC is a low-cost commercially available polyanionic polysaccharide derivative of cellulose that has been employed in pharmaceuticals and cosmetics [14]. Due to its biocompatibility and biodegradability, it is considered as a good candidate for drug delivery applications [15,16]. PAH was selected as the polycation in this study, as biomaterial applications of multilayer films containing PAH have previously been reported [17]. $CaCO_3$ particles were chosen as microtemplates, as they are non-toxic and easily reproducible. They can be dissolved by complexation with ethylenediaminetetraacetic acid (EDTA) or under slightly acidic conditions [18].

In this paper we report a novel microcapsule system with controllable loading and release capabilities for protein delivery applications. The stimuli-responsive multilayer hollow microcapsules have been fabricated by the sequential layer-by-layer electrostatic assembly technique onto templates ($CaCO_3$) with CMC as the polyanion and PAH as the polycation, respectively. We have investigated the spontaneous deposition of BSA into CMC/PAH capsules based on "charge controlled attraction" mechanism by tailoring the pH induced permeability of the capsule wall. We have also shown that the loading and subsequent release could be tuned by factors such as feeding concentration and loading pH.

2. Experimental

2.1. Materials

PAH (M_W = 70 kDa), CMC (M_W = 70 kDa), sodium carbonate, calcium chloride, ethylenediaminetetraacetic acid (EDTA), FITC–dextran (M_W = 70 kDa), FITC–BSA, BSA (M_W = 66 kDa) and phosphate buffered saline (PBS) were all purchased from Sigma–Aldrich and used without any further purification. The water used in all experiment was obtained from Milli-Q system with resistivity greater than 18 MΩ cm. All pH adjustments were done with 0.1 M HCl or 0.1 M NaOH.

2.2. Preparation of $CaCO_3$ microparticles integrated with CMC

$CaCO_3$ microparticles doped with CMC, abbreviated as $CaCO_3$ (CMC), were synthesized by mineralization of Ca $(NO_3)_2$ and Na_2CO_3 solutions in the existence of CMC [19]. Briefly, 100 mL 0.025 M Ca $(NO_3)_2$ solution was mixed with 2 mL of 5% CMC solution, into which 100 mL Na_2CO_3 solution was rapidly poured under ultrasonication. After 15 min the particles were filtered and washed using a membrane filtration apparatus equipped with a cellulose filter having pore size of 0.45 μm and dried in air.

2.3. Microcapsule preparation

Multilayered microcapsules were fabricated using electrostatic layer-by-layer (LBL) self-assembly. PAH and CMC were alternately assembled onto $CaCO_3$ (CMC) microparticles. $CaCO_3$ (CMC) colloidal particles were rinsed twice with pH adjusted water solution and then incubated in 1 mg/mL PAH solution for 10 min. The particles were then washed three times by immersing in pH 5 water for 1 min followed by centrifugation at 3000 rpm for 3–4 min. In the next step, the microparticles were incubated in 0.5 mg/mL CMC solution for 10 min, followed by three washings. Repeating the deposition of PAH and CMC led to the formation of desired number of PAH–CMC multilayers on $CaCO_3$ (CMC) microparticles. After completion of the multilayer assembly, the calcium carbonate core was dissolved using 0.2 M EDTA solution at pH 7. After 15 min, the capsules were centrifuged and resuspended in fresh EDTA followed by washing in pH adjusted deionized water for 3 times.

2.4. Loading of BSA into microcapsules

Capsule suspension was centrifuged at 6000 rpm for 6 min and the supernatant was removed. Then 0.2 mL capsule suspension was mixed with 0.8 mL of BSA solution at different loading conditions such as pH and feeding concentration. The amount of BSA loaded inside the microcapsules was estimated from the difference between input amount and amount in the supernatant after loading process. The BSA content in the supernatant was determined directly at 280 nm using a ND-1000 UV–vis spectrophotometer (Nanodrop Technologies, USA). All the data are averaged from 3 parallel experiments.

The important loading parameters such as loading capacity [20] (L), capsule interior concentration [21] [C_{in}] and loading efficiency [E] were calculated by using the following formulae.

$$\text{Loading capacity}\,(L) = \frac{[C]N_A}{M_W[N]} \tag{1}$$

$$\text{Capsule interior concentration}\,[C_{in}] = \frac{[c]}{V_1^*[N]^*} \tag{2}$$

$$\text{Loading efficiency}\,[E] = \frac{[c]}{c_f} \tag{3}$$

where [c] is concentration of loaded protein in mg/mL, M_W is the molecular weight of the loaded protein in g/gmol, [N] is number of capsules per liter, N_A is the Avogadro's number, V_1^* capsule is the volume of one microcapsule, $[N]^*$ is number of capsules/mL and C_f is concentration of feeding solution in mg/mL. The number of microcapsules was determined using a hemocytometer.

2.5. BSA release

The loaded capsules ($3.4 \pm 0.1 \times 10^6$ capsules) were incubated in 1 mL of PBS or water at pH 7.4 in an eppendorf tube. Then the eppendorf tube was placed in a shaker at 100 rpm/min, 37 °C for 30 min. 0.65 mL of the supernatant was taken out each time from the encapsulated BSA, whilst supplementing the same volume of water to keep the total volume constant at 1 mL. The amount of BSA in supernatant solution was measured directly at 280 nm by UV–vis spectroscopy.

2.6. ζ-Potential measurements

ζ-Potential of particles during the coating process and of the empty capsules after core dissolution was measured in triplicate at room temperature using the Zetasizer (Malvern Instruments, UK).

2.7. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of dried capsules mixed with KBr were acquired using a Nicolet 5700 FT-IR spectrometer (Thermo Electron Corporation, USA). The spectra of CMC, PAH and empty capsules were collected at 4 cm^{-1} resolution, 500 scans with frequency range between 4000 cm^{-1} and 500 cm^{-1}.

2.8. Scanning electron microscopy (SEM)

The extent of core dissolution and morphology of capsules was studied by SEM (FEI-SIRION, Eindhoven, The Netherlands). A droplet of the aqueous capsule suspension was dried overnight on a silicon wafer in a desiccator at room temperature. Samples were analyzed after sputtering a thin 10 nm gold layer on the sample.