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ScienceDirect
Acta Biomaterialia 5 (2009) 2122–2131
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A study of properties of "micelle-enhanced" polyelectrolyte capsules: Structure, encapsulation and in vitro release

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Received 18 September 2008; received in revised form 7 January 2009; accepted 26 January 2009
Available online 4 February 2009

Abstract

"Micelle-enhanced" polyelectrolyte capsules were fabricated via a layer-by-layer technique, templated on hybrid calcium carbonate particles with built-in polymeric micelles based on polystyrene-b-poly(acrylic acid). Due to the presence of a large number of negatively charged micelles inside the polyelectrolyte capsule, which were liberated from templates, the capsule wall was reconstructed and had properties different to those of conventional polyelectrolyte capsules. This type of capsule could selectively entrap positively charged water-soluble substances. The encapsulation efficiency of positively charged substances was dependent on their molecular weight or size. For some positively charged compounds, such as rhodamine B and lysozyme, the concentration in the capsules was orders of magnitude higher than that in the incubation solution. In addition, in vitro release study suggested that the encapsulated compounds could be released through a sustained manner to a certain degree. All these results point to the fact that these capsules might be used as novel delivery systems for some water-soluble compounds.

Keywords: Functionalized templates; Self-assembly; Polyelectrolyte capsules; Water-soluble substances; Induced deposition

1. Introduction

Hollow capsules have attracted great interest in recent years for their potential applications in medicine, pharmaceuticals, artificial cells or viruses, cosmetics, food and catalysts [1–5]. Among the preparation methods, layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes (PE) onto removable colloidal particles is a widely employed technique to prepare novel hollow nano and microcapsules [6–9]. A variety of substrates with a charged surface has been used as templates for the formation of multilayer capsules, such as weakly cross-linked melamine formaldehyde (MF) colloidal particles, organic and inorganic crystals, silicon particles, polystyrene latices, metal nanoparticles or nanorods, and biological templates [10–13]. In most of these systems, the templates only supply a physical support, and the cores are routinely decomposed completely after the desirable multilayers have been deposited.

Many attempts have been made to encapsulate drugs, proteins, dyes, genes, minerals, and enzymes into hollow capsules [14–16]. By varying the solvent and pH, or salt exposure, the shell permeability of PE microcapsules can be modulated, and the candidate compounds can then be encapsulated into hollow capsules [17,18]. In addition, by modulating the solubility of model substances through solvent or pH variation, or in situ polymerization of monomers, candidate compounds can be encapsulated into polyelectrolyte capsules as well [19]. However, for these

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doi:10.1016/j.actbio.2009.01.045

loading procedures, precipitation or polymerization inevitably occurs both in the capsule and in the bulk simultaneously, leading to difficulties for purification and low encapsulation efficiency. Since the loading amount is limited by the bulk concentration, encapsulation with high efficiency, especially for water-soluble substances, is impossible.

To circumvent this problem, many ingenious experiments have been designed to encapsulate substances into PE capsules with high efficiency. By pre-depositing the candidate substances on porous templates such as porous $CaCO_3$ or mesoporous silica spheres [13,20–25] and then fabricating PE multilayers on these templates, high efficiency encapsulation could be achieved for PE capsules after the disposal of templates. In addition, PE capsules templated on protein–calcium carbonate co-precipitate can achieve high efficiency encapsulation for protein due to the high protein content in the co-precipitate after removal of the calcium carbonate [26]. Furthermore, it was found that water-soluble substances could spontaneously deposit into the MF-based capsules due to the presence of MF residues in the capsule interior, which can interact with poly(styrene sulfonate sodium) salt molecules from the capsule wall, forming a negatively charged complex [27]. Besides serving as physical support, these templates have a great influence on the encapsulation properties of resultant PE capsules, which inspired us to design special templates to impart resultant PE capsules with modified properties [28,29].

In our previous study, by synthesizing novel templates, i.e., acid-resistant hybrid $CaCO_3$ spheres, in the presence of block copolymer polystyrene-b-poly (acrylic acid), "micelle-enhanced" PE microcapsules were obtained by the LbL technique [30,31]. The wall of these "micelle-enhanced" PE capsules was reconstructed and was different from that of conventional PE capsules. In this study, we investigated the encapsulation capability and dynamics of "micelle-enhanced" capsules for different positively charged substances. The fluorescence recovery after photo-bleaching (FRAP) technique was employed to demonstrate the form of the encapsulated substances. Qualitative and quantitative studies of the encapsulated substances evidenced the high encapsulation capacity of "micelle-enhanced" PE microcapsules for water-soluble substances. Based on these experiments, the encapsulation mechanism was reasonably elucidated. Furthermore, the release behaviors of the filled PE capsules were investigated as well.

2. Materials and methods

2.1. Materials

Amphiphilic block copolymer polystyrene-b-poly (acrylic acid) (PS-b-PAA) ($M_w = 12{,}500$, the molar ratio of styrene to acrylic acid is approximately 2.0) was obtained from Rohm & Hass Company (Philadelphia, USA). Poly(styrene sulfonate sodium salt) (PSS) ($M_w = 70{,}000$) and poly(allylamine hydrochloride) (PAH) ($M_w = 70{,}000$) were purchased from Aldrich (Munich, Germany). Fluorescein isothiocyanate (FITC) was purchased from Sigma (St. Louis, USA). Rhodamine B and fluorescein sodium were supplied by Shanghai Zhibo Chemical Company (Shanghai, China). PAH and lysozyme were labeled with FITC in our laboratory (the molar ratio of PAH, lysozyme or albumin to FITC is 1:4). All the other reagents were commercially available and used as received.

2.2. Preparation of hybrid $CaCO_3$ microparticles and "micelle-enhanced" PE capsules

Hybrid $CaCO_3$ particles with desirable size were prepared by modulating PS-b-PAA concentration in the reaction system, and "micelle-enhanced" PE capsules were fabricated according to Ref. [30]. In brief, 20 ml $CaCl_2$ aqueous solution (2 mol l^{-1}) was rapidly added into 200 ml Na_2CO_3 aqueous solution (0.2 mol l^{-1}) containing 0.24 mmol l^{-1} PS-b-PAA and stirred for 30 min. The precipitate was filtered through 0.45 μm membrane and thoroughly rinsed with deionized water. Hybrid microparticles with the diameter of 8.4 μm were thus obtained. PAH and PSS were in turn absorbed on hybrid $CaCO_3$ microparticles from 2 mg ml^{-1} store solutions. After the adsorption of 12 layers, the hybrid cores were dissolved in trisodium EDTA solution. The resultant microcapsules were washed four times with deionized water and stored in the centrifuge tubes for further experiment.

2.3. Study on the selective encapsulation

Five samples of capsule suspension (100 μl each one) were mixed with 400 μl (the concentration was 1000 μg ml^{-1}) of rhodamine B solution, FITC–PAH solution, FITC–albumin solution, FITC–lysozyme solution and sodium fluorescein solution, respectively, and then incubated for 2 h at room temperature at neutral pH. After centrifugation, the precipitate was rinsed with deionized water five times to remove non-encapsulated substances. The microcapsules were re-suspended in deionized water and observed by confocal laser scanning microscopy (CLSM, LSM510, Zeiss, Germany) immediately. All the supernatant was collected together for the quantification of entrapped substances through UV–vis measurement. The encapsulation efficiency (EE) of PE capsules for the five model compounds was measured as well:

$$EE = \frac{(c_0 - c_n)}{c_0} \times 100\% \quad (1)$$

where EE represents the encapsulation efficiency for various candidate substances under the same condition, c_0 is the initial concentration of candidate substances in the incubation solution (μg ml^{-1}), while c_n is the solution concentration after incubation (μg ml^{-1}). Both c_0 and c_n can be directly determined through UV–vis measurement.

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