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Heat-driven size reduction of biodegradable polyelectrolyte multilayer hollow capsules assembled on $CaCO_3$ template

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ABSTRACT

Aiming to explore elevated temperatures as a tool for miniaturization of biodegradable polymer multilayer capsules, assembled on spherical vaterite micron- and submicron-sized particles, we subject the shells composed of dextran sulfate (DS) and poly-L-arginine (Parg) to a heat treatment. Changes of the capsule size are studied at various temperatures and ionic strengths of the continuous phase. Unlike some synthetic polymer multilayer shells (their response to heat treatment depends on the number of layers and their arrangement), the biodegradable Parg/DS capsules exhibit size reduction and profound compaction regardless of their initial size, number of polymer layers and polymer layer sequence. The capsule response to heat is stable at ionic strengths of the continuous phase not exceeding 0.1 M NaCl.

1. Introduction

The use of micro- or nanocarriers delivering the encapsulated actives to the site of action is now a common practice in several biological applications, *e.g.*, biomedicine, biotechnology, cosmetics, and functional foods. Among delivery systems of high potential, polyelectrolyte multilayer carriers represent a powerful tool for controlled release of biologically active payloads. This tool is adaptive and flexible to match the relevant available release stimuli, *e.g.* action of enzymes [1–4], change of redox potential [5–7], treatment with glucose [8] and remote capsule collapse by physical force (ultrasound [9], magnetic field [10,11], and near infrared light irradiation [12]). A simple and efficient assembly method enables their well-recognized stability and high efficacy of loading with a wide range of bioactive compounds and drugs [13–18].

The size of delivery vehicle is one of the most important parameters that requires optimization in order to fit the specific biological needs. A large number of published research results claim an observed biological effect of drugs delivered by the polymeric multilayer capsules with sizes between 2 and 5 μm *in vitro*. However, *in vivo* applications are constantly demanding further miniaturization of the applied delivery systems to enhance the therapeutic efficacy and bioavailability of the payload [19]. The size of a layer-by-layer (LbL)-formed capsule is predetermined by the size of its sacrificial template. Different sacrificial templates, including those potentially available on the submicron- and nanoscale, have been applied for hollow multilayer capsules, *i.e.*, particles of melamine formaldehyde (MF) [20], polystyrene (PS) latex [21,22], silicon dioxide (SiO_2) [23,24], gold [25], and some naturally occurring substances, *e.g.* biological cells [26]. Each of these templates has certain limitations that are mainly associated with either toxicity or/and dissolution conditions. For instance, decomposition of gold or silica templates implies respective use of potassium cyanide or hydrofluoric acid. These substances are toxic and require extreme caution upon handling; in addition, they could cause contamination of the final formulation. Dissolution of cores usually creates an osmotic pressure that often destroys the polyelectrolyte shell. This effect is especially prominent for biocompatible capsules made of natural polymers. In addition, MF and PS templates are known for their incomplete degradation, thus the capsules built on such templates will face restrictions upon application *in vivo*. From this perspective, calcium carbonate particles appear to be a far better template, considering their biocompatibility, non-toxic nature, and mild conditions required for their dissolution [27].

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The vaterite framboids [28] that possess a well-developed porous structure are the most suitable polymorphs of $CaCO_3$ for the LbL encapsulation purposes. Vaterite was successfully applied for pre-loading the polymeric multilayer capsules with proteins [29], enzymes [30], nucleic acids including highly unstable molecules of mRNA [31], hydrophilic and hydrophobic drugs [13]. [18,32,33], However, our previous research on controlled preparation of vaterite revealed that the smallest size of the obtained particles falls in the range of 400–500 nm [34]. Therefore, to achieve the vaterite templated capsules of smaller sizes, additional measures to reduce the capsule size in the post-preparation stage have to be considered.

Elevated temperature is known to cause tightening of the polymeric multilayer infrastructure that leads to a drastic reduction in capsule size. Thus, treatment of the formed hollow capsules by heat has high potential for exploration as a strategy for further compaction of these delivery systems. The effect of temperature on the capsule size was previously studied and reported for the capsules composed of purely synthetic polymer pairs, *e.g.*, poly(allylamine)/poly(sodium 4-styrenesulfonate) (PAH/PSS) and polydiallyldimethylammonium/poly(sodium 4-styrenesulfonate (PDADMAC/PSS) [35,36]. The observed changes, such as shrinking/swelling and the level of compaction, were found dependent on the polymeric pair and the number of layers in the polymeric assembly. The capsule response to heat was generally explained by the increase in the kinetic mobility of polymer chains, causing possible rearrangement and/or formation of additional ion pairs between the polymer counterparts that are fitted with pending groups of opposite charges. We reason that a direct comparison of the data obtained for multilayer capsules, composed from synthetic polymers, to those assembled by LbL technique from biopolymer pairs would not be accurate. The kinetic mobility in the multilayer complexes of biopolymers will not be affected to the same degree by the action of heat, as it is observed for polymeric structures in capsules made of synthetic polyolefins. High molecular mass biopolymers with well-developed branched stricture (*e.g.* polysaccharides) together with their counterparts, proteins, possessing rigid, highly arranged (due to the repetitive hydrogen bonding) chains (*e.g.* poly(homo)amino acids) are expected to react to the heating much differently. Thus, one can expect a far more different influence of heating on the macrostructure of biodegradable multilayers, and especially on the associated size change.

For this study, we selected a polymer pair polyarginine (Parg) – dextran sulfate (DS) (Fig. S1, Supporting information) which is known for its biodegradability [2,37], good structural integrity, and low cytotoxicity [1,38,39]. Here we present detailed research on the response to heat of the Parg/DS capsules assembled in the $CaCO_3$ sacrificial template. The capsule size is examined while varying temperature, duration of the heat exposure, capsule thickness, capsule original size and ionic strength of the continuous phase. The differences in thermal behavior of either biopolymer- or synthetic polymer-based multilayer capsules are also discussed in the paper.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and used as received without further purification. Calcium chloride dihydrate ($CaCl_2 \times 2H_2O$), anhydrous sodium carbonate (Na_2CO_3), glycerol, ethylenediaminetetraacetic acid (EDTA), dextran sulfate sodium salt (M_W = 9000–20000), poly-L-arginine hydrochloride (M_W > 70,000), sodium chloride (NaCl), rhodamine 6 G (MW = 479), Tetramethylrhodamine isothiocyanate (TRITC)-dextran, MW = 40,000), and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich; FBS-DMEM medium was purchased from Gibco Thermo Scientific. Deionized water from a three-stage Milli-Q Plus purification system was used in the experiments.

2.2. Vaterite synthesis

Spherical micron-sized vaterite particles were synthesized by stirring equal volumes of 1 M $CaCl_2$ and 1 M Na_2CO_3 aqueous solutions for 20 s. Submicron-sized $CaCO_3$ vaterite was synthesized as described elsewhere [34]. In brief, equal volumes of 0.1 M $CaCl_2$ and 0.1 M $NaCO_3$ aqueous solutions were mixed with glycerol in a volume ratio of 1:1:5. After 60 min of continuous stirring (500 rpm) at room temperature, the suspension was centrifuged (3000 *g*, 5 min), and the sediment was washed with deionized water three times. The particles were dried at 70 °C under vacuum and stored in a dry form.

2.3. Preparation of hollow polymer multilayer capsules

Spherical vaterite $CaCO_3$ particles were further subjected to the LbL coating with polyelectrolytes. The particles were dispersed in poly-L-arginine (Parg) solution (2 mg/mL, 0.5 M NaCl) under vigorous shaking for 15 min at room temperature. After the Parg deposition step, the particles were separated from the supernatant by centrifugation and washed with water twice to remove the excess of polymer. Then the particles were coated with a negatively charged layer of dextran sulfate (DS) under the same conditions as described above for Parg adsorption, using DS solution (2 mg/mL, 0.5 M NaCl). Consecutive deposition of the Parg and DS layers was carried out by repeating these steps in a sequence until the desired number of layers (from 4 to 9) were achieved. The formation of the Parg/DS multilayer was followed by ζ-potential measurements (Fig. S2, Supporting information). ζ-Potential charge reversal indicates adsorption of the consecutive polymeric layer. Hollow capsules were produced by dissolving the $CaCO_3$ core in a buffered EDTA solution (pH 5). For this purpose, thoroughly washed $CaCO_3$/Parg/DS core/shell particles were mixed with 2 mL of 0.5 M EDTA solution and kept under constant shaking until the suspension became visibly more transparent. The capsules were then collected by centrifugation at 9000*g* for 5 min. The dissolution step was repeated if necessary to ensure complete removal of $CaCO_3$.

2.4. Size reduction of the hollow polymer multilayer capsules

The resulting hollow capsules were further subjected to thermal treatment. For this purpose, 300 µL of the capsule aqueous suspension was rotated on orbital shaker (1000 rpm) at either 50, 60, 70, 80, or 90 °C. The duration of the heat exposure was varied at four time points: 15, 30, 60, and 120 min. The process of the capsule assembly and heat treatment is depicted in Fig. 1.

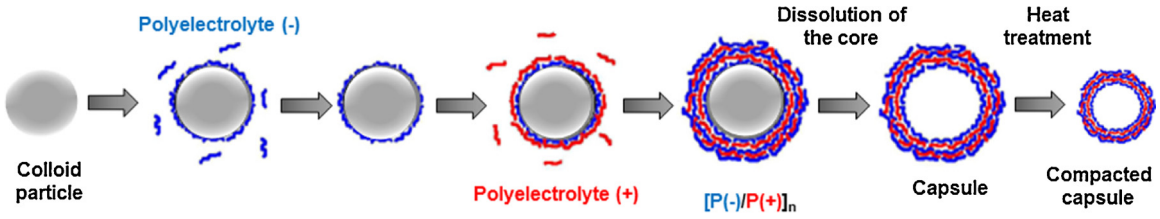

Fig. 1. The scheme of the polyelectrolyte multilayer capsule assembly and heat treatment process.