

Chitosan/dextran multilayer microcapsules for polyphenol co-delivery



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ARTICLE INFO

Article history:

Received 16 September 2014

Accepted 21 October 2014

Available online 23 October 2014

Keywords:

Nanostructured polymeric capsules

Layer-by-layer

Controlled release

Polyphenols

Acidic environment

ABSTRACT

Polysaccharide-based nanostructured polymeric microcapsules were fabricated by the electrostatic layer-by-layer self-assembly technique and used to encapsulate mixtures of four different polyphenols in order to achieve their controlled release. The real-time fabrication of the dextran/chitosan multilayer was monitored by quartz crystal microbalance with dissipation monitoring, and the morphology of the nanostructured polymeric capsules was characterized by scanning electron microscopy. The polyphenol encapsulation was obtained by reversible permeability variation of the capsule shell in ethanol:water mixtures. The loading efficiency in different water:ethanol mixtures and the release rate in acidic conditions were characterized by UV spectroscopy and HPLC. The higher loading efficiency was obtained with an ethanol:water 35:65 phenolic solution, equal to $42.0 \pm 0.6\%$, with a total release of 11.5 ± 0.7 mg of total polyphenols per $11.3 \mu\text{L}$ of microcapsules after 240 min of incubation in acidic environment. The results suggest that polysaccharide-based capsules can be successfully used to encapsulate and release low water-soluble molecules, such as polyphenols.

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1. Introduction

Polyphenols are secondary metabolites present in plants. They are a large family of substances, ranging from simple molecules to complex structures [1]. These compounds show a wide spectrum of biological properties such as antioxidant, anti-inflammatory, antibacterial and antiviral activities [2]. Antioxidant properties make polyphenols potential therapeutic agents against serious diseases, like cancer, diabetes and cardiovascular disorders [3–8], acting against reactive oxygen species generated by exogenous chemicals or endogenous metabolism [9] and preventing cell damages caused by oxidative stress [10].

Several limitations have been associated with low bioavailability of polyphenols, including limited stability in environmental conditions, such as temperature, light, moisture, pH, oxygen concentration [11], low water solubility and rapid catabolism in the upper gastrointestinal tract and liver [12] and fast excretion through urinary system [13]. *In vitro* studies have shown that biological effects of polyphenols are extremely dose dependent and are evidenced at much higher concentrations than those present in natural sources [14].

Abbreviations: CAE, caffeic acid equivalents; CHI, chitosan; DEX, dextran sulfate; LE, loading efficiency; TPC, total phenolic concentration; NPC, nanostructured polymeric capsules; QCM-D, quartz crystal microbalance with dissipation monitoring; SEM, scanning electron microscopy.

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The development of effective encapsulation strategies of such molecules is therefore most desirable in order to fully exploit their therapeutic potential. Encapsulation in micro/nanoscale delivery systems can improve polyphenols half-life *in vivo*, preserving their biological activities, and can enhance their bioavailability. Moreover, encapsulation in a nanoengineered carrier can be used in order to achieve targeted delivery of the molecules into the diseased tissue with a controlled release profile [15]. Several encapsulation strategies for polyphenols have been proposed so far, mainly based on the use of liposomes, micelles, emulsions and spray drying techniques [16]. Engineering the structure, and thus the function, of the delivery system at the nanoscale resolution plays a pivotal role in the design of new successful treatment regimes. Moreover, cost is an important factor for the industrialization of such nanoformulation. In this framework, nanostructured polymeric capsules (NPC), obtained by the electrostatic layer-by-layer self-assembly technique, have been shown to possess great potentialities [17]. NPC are fabricated by the alternate adsorption of oppositely charged polyelectrolytes onto the surface of micro/nanoscale templates, usually carbonate particles [18]. Once the polyelectrolyte multilayer which constitutes the capsules shell has been deposited, the template is removed by dissolution in acidic medium or by chelating agents [19, 20]. By this technique it is possible to fabricate hollow polyelectrolyte capsules whose shell thickness ranges from few nanometers to tens of nanometers and with a predetermined composition, structure and thus functionality. An interesting property of such systems is the possibility to vary shell permeability as a consequence of a specific stimulus

[21–25], usually pH, in order to load and, if required, to release cargo molecules. The assembly process is versatile and not very expensive, requiring only simple laboratory equipment. Therefore, NPC can be regarded as a very promising carrier for industrial scale-up.

The main requirement to be satisfied for drug delivery systems is the use of fully biocompatible and biodegradable carriers. In this respect, the use of biopolymers having well characterized physico-chemical properties is highly desirable. Natural polysaccharides have received great attention in the last few years, due to their unique features for applications in the field of drug delivery systems [26,27]. Polysaccharides are highly stable, safe, non-toxic and biodegradable, and can be easily chemically modified, resulting in a wide range of derivatives exhibiting different characteristics. Moreover polysaccharides, and specifically chitosan, have natural bioadhesive properties towards biological tissues that could prolong the residence time and therefore increase the absorbance of loaded drugs [28]. Chitosan, a copolymer of glucosamine and N-acetyl glucosamine, is a polycationic, biocompatible and biodegradable natural biopolymer mainly derived from the outer shells of crustaceans such as crabs and shrimps. Chitosan has different functional groups that can be modified with a wide range of ligands. Because of its properties, chitosan has great potential in biomedical applications, including drug delivery and tissue engineering [29]. Due to its cationic nature, chitosan is a good candidate for the layer-by-layer technique.

One of the polysaccharides which displays complexing properties with chitosan is the polyanion dextran sulfate, which is obtained by the esterification of dextran using sulfuric acid. Several dextran sulfate/drug conjugates have been proposed as drug delivery systems [30].

In the present work, we describe the fabrication of biocompatible and biodegradable NPC, composed by cationic chitosan deposited in alternation with anionic dextran sulfate, and their use for the encapsulation of an ensemble of polyphenolic molecules for a synergistic effect. The deposition process and the structural properties of the chitosan/dextran sulfate multilayer were characterized by quartz crystal microbalance with dissipation monitoring (QCM-D). Then the assembly procedure was used for the deposition of the multilayers onto the surface of CaCO_3 microparticles, followed by their removal under treatment with the chelating agent EDTA. The hollow NPC were then used for the encapsulation of polyphenols by means of the reversible permeability increase of their shell in ethanol: water mixtures containing polyphenols [31]. Loaded and unloaded NPC were structurally characterized by scanning electron microscopy. The polyphenol release in simulated gastric environment was characterized by means of UV–vis spectroscopy and HPLC. Finally, the influence of the thickness of the NPCs shell on the release rate was evaluated.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol, acetic acid, acetonitrile, Folin–Ciocalteu reagent, medium MW chitosan (CHI), dextran sulfate sodium salt (DEX) from *Leuconostoc* spp. (MW 9000–20,000), NaCl, CaCO_3 , Na_2CO_3 , ethylenediaminetetraacetic acid (EDTA) and standards of tyrosol, caffeic acid, vanillic acid and *p*-coumaric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The polysaccharides were used as received. CHI and DEX solutions were prepared with a concentration of 0.5 mg/mL in NaCl 0.5 M. Chitosan was dissolved by addition of acetic acid to a final concentration of 0.3% (v/v) under continuous stirring overnight.

Standards of tyrosol, caffeic acid, vanillic acid and *p*-coumaric acid were mixed and dissolved in ethanolic (20%, 35% and 50% v/v) solutions at a concentration of 2.5 mg/mL for each molecule.

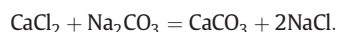
2.2. Quartz crystal microbalance with dissipation monitoring

The build-up of DEX/CHI multilayer was monitored by QCM-D (QCM-Z500, KSV Instruments, Helsinki, Finland). This technique has been extensively described [32,33], and allows to evaluate simultaneously the normalized resonant frequency (Δf) and energy dissipation shifts (ΔD) [32]. A quartz crystal with gold plated polished electrodes is excited at its fundamental frequency (5 MHz) and at the 3rd, 5th, 7th, 9th and 11th overtones (12, 25, 35, 45 and 55 MHz). As the mass is deposited onto the crystal surface, the oscillation frequency decreases. If the deposited mass is rigidly attached to the crystal, the frequency decrease is proportional to the mass and can be calculated using the Sauerbrey equation [34]. However, for viscoelastic materials the deposited mass introduces a dissipative energy damping. Using a Voigt-based model [35], the QCM-D response of a viscoelastic material can be modeled and the properties of added layers such as mass, density and thickness can be obtained. In this model, the adsorbed film is represented by a single Voigt element consisting of a parallel combination of a spring and dashpot to represent the elastic (storage) and inelastic (damping) behavior of a material, respectively.

Before adsorption, the quartz crystals were cleaned with H_2SO_4 at 150 °C for 20 min followed by washing in pure water. A PTFE liquid chamber with a volume of 2 mL was used in the experiments. Polysaccharide solutions were alternatively introduced into the measurement chamber and left in contact with the quartz crystal. After each adsorption step, pure water was poured into the chamber and left in contact with the crystal for 1 min in order to remove the unabsorbed polysaccharides. The data analysis was performed using the QCM Impedance Analysis software (KSV Instruments, version 3.11).

2.3. NPC preparation

NPC were assembled onto calcium carbonate sacrificial microparticles (6 μm in diameter), obtained by mixing calcium chloride and sodium carbonate solutions according to the reaction [36,37]:



10^8 CaCO_3 microparticles were covered by successively deposited layers of anionic DEX and cationic CHI. Polysaccharides were left to adsorb onto the microparticle surface for 20 min, after each deposition step the dispersion of covered particles was centrifuged (2500 rpm for 5 min) and the precipitated covered particles were separated from the solution. These particles were washed three times in pure water, with successive centrifugation and separation steps. Four or eight bilayers were deposited onto the surface of the microparticle. Microparticles were then dissolved by their dispersion in EDTA solution at a concentration of 0.5 M at pH 7 followed by three washing steps in pure water.

Knowing the diameter and the number of the prepared NPC, the internal volume of the NPC batch was calculated, resulting equal to $11.30 \pm 0.90 \mu\text{L}$.

2.4. NPC loading

The phenolic concentrations in the loading solutions were determined using the Folin–Ciocalteu assay [38]: 0.2 mL of diluted solution and 0.5 mL of Folin–Ciocalteu reagent were added to 4.8 mL of deionized water and, after mixing, 1 mL of a 20% Na_2CO_3 solution was added. Deionized water was added in order to reach a final volume of 10 mL. Solutions were mixed and incubated at room temperature in dark conditions for 1 h. Sample aliquots were used for the determination of total phenolic concentration (TPC) using a UV–vis spectrophotometer (Perkin Elmer, Wellesley, USA) at a wavelength of 725 nm. Concentrations were expressed as mg of caffeic acid equivalents (CAE) per mL. Caffeic acid was chosen as a standard due to its wide use as reference when working with phenolic compounds [39–41]. Absorbance

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