International Journal of Biological Macromolecules xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Hollow chitosan/alginate nanocapsules for bioactive compound delivery

Q1 Melissa C. Rivera, Ana C. Pinheiro, Ana I. Bourbon, Miguel A. Cerqueira, António A. Vicente*

Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

Article history:
Received 20 October 2014
Received in revised form 19 February 2015
Accepted 3 March 2015
Available online xxx

Keywords:
Biodegradable polysaccharides
Multilayer
Chitosan
Alginate
Functional compounds

ABSTRACT

This work aimed at the development of biodegradable nanocapsules as carriers of two bioactive compounds, 5-aminosalycilic acid and glycomacropeptide. Nanocapsules were produced through layer-by-layer (LbL) deposition of chitosan (CH) and alginate (ALG) layers on polystyrene nanoparticles. The bioactive compounds were incorporated on the third layer of the nanocapsules being its encapsulation efficiency and release behaviour evaluated.

The LbL deposition process, stability, morphology and size of the multilayer nanocapsules were monitored by means of zeta potential and transmission electron microscopy (TEM). The bioactive compounds release from the CH/ALG nanocapsules was successfully described by a mathematical model (linear superimposition model – LSM), which allowed concluding that bioactive compounds release is due to both Brownian motion and the polymer relaxation of the CH/ALG layers.

Final results demonstrated that the synthesized LbL hollow nanocapsules presented spherical morphology and a good capacity to encapsulate different bioactive compounds, being the best results obtained for the system containing 5-aminosalycilic acid (with an encapsulation efficiency of approximately 70%).

CH/ALG multilayer nanocapsules could be a promising carrier of bioactive compounds for applications in food and pharmaceutical industries.

1. Introduction

Q2

Over the last decade, the potential use of nanotechnology has been growing in industries such as food [1] and pharmaceutical [2]. The design of micro-/nanostructures has been receiving much attention, being these structures widely applied as carrier/delivery systems of bioactive compounds (BCs) [3], where the BC benefits from an encapsulation procedure that slows down or/and prevents degradation processes until the product is delivered at the desired target (e.g. colon, stomach and small intestine) [4], thus enhancing their therapeutic effect.

Layer-by-layer (LbL) technique turned into a widely used method for the preparation of different multilayer nanostructured systems [5]. This technique consists of the alternated deposition of oppositely charged polyelectrolytes around a charged template. Multilayer nanocapsules can be obtained using a colloidal template, such as polystyrene nanoparticles, melamine formaldehyde and gold nanoparticles [6,7]. Then, at the end of the LbL deposition process, the templates is removed to obtain hollow capsules [3], which can be applied in various fields [8]. One example is their use to carry, protect and deliver bioactive compounds (BCs) or functional ingredients (e.g. drugs, antimicrobials, antioxidants and flavourings) to a specific site of action [9]. Also, this technique is an inexpensive, highly adaptable and easy solution-based assembly method, thus allowing materials to be designed and assembled with tailored properties and nanoscale precision [10].

Different strategies have been envisaged for bioactive compounds loading into LbL multilayer nanocapsules: (i) preloading the BC either in or onto the template, (ii) incorporating the BC with the layers during multilayer assembly, or (iii) post-loading the cargo into preformed capsules [10]. Glycomacropeptide (GMP) is a C-terminus portion (f 106–169) of kappa-casein (milk protein) released in whey during cheese making by the action of chymosin [11]. This peptide is known for its several beneficial biological properties, such as prevention of dental caries, zinc absorption and bacterial inhibition [12]. 5-Aminosalicylic acid (5-ASA) is an anti-inflammatory drug that has been employed for several years in the treatment and remission of inflammatory bowel disease (IBD),

* Corresponding author.
E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

http://dx.doi.org/10.1016/j.ijbiomac.2015.03.003
0141-8130/

among which ulcerative colitis and Crohn's disease are the most diffuse and important [13].

In this study, GMP and 5-ASA, were used as bioactive compound models and incorporated in nanocapsules during multilayer assembly. Moreover oppositely charged biopolymers such as chitosan (CH) and alginate (ALG) have been used in the formation of hollow nanocapsules. Chitosan is a linear cationic polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) and it is obtained via alkaline deacetylation of chitin [14]. Alginate is a natural anionic polysaccharide, soluble in water and extracted from marine brown algae (Phaeophyta) by treatment with aqueous alkali solutions [15]. This polysaccharide is now known for being a whole family of linear copolymers containing blocks of uronic acids, L-guluronic acid (G) and D-mannuronic acid (M), where the relative amount of the uronic acid monomers as well as their sequential arrangement along the polymer chain differs widely, depending on the origin of the alginate [16].

There has been a great interest in the study of the behaviour of LbL nanocapsules as drug delivery systems in different areas [17]. Therefore, it is of significant importance to develop nanocapsules capable of encapsulating different BCs and evaluate their behaviour during BC release.

In order to achieve this goal, the main objectives of this work were the development and characterization of CH/ALG multilayer hollow nanocapsules trough LbL deposition and evaluated their potential use as carrier of different BCs. GMP and 5-ASA (used BCs) were incorporated into the nanocapsules and their encapsulation efficiency and release behaviour was assessed.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree ≥95%) was purchased from Golden-Shell Biochemical Co., Ltd. (Zhejiang, China) and sodium alginate from Manutex RSX, Kelco International, Ltd. (Portugal). 5-Aminosalicylic acid with a mass fraction purity of 99% was purchased from Sigma–Aldrich (St. Louis, USA). Commercial glycomacropeptide (GMP) was obtained from Davisco Food International, Inc. (Le Sueur, MN, USA). Sodium hydroxide and hydrochloric acid at 37% were obtained from Riedel-de Haen (Germany). Lactic acid (90%) was purchased from Merck (Germany). Hanks balanced salt solution (HBSS) was obtained from Invitrogen (Gibco, Invitrogen Corporation, Paisley, UK). Polystyrene nanoparticles were purchased from Polysciences, Inc. (Warrington, PA, USA). All samples were prepared with distilled water purified to a resistance of 15 MΩ cm.

2.2. Layer-by-Layer assembly

2.2.1. Preparation of polyelectrolyte solutions

Polyelectrolyte solutions were prepared dissolving sodium alginate (ALG) in distilled water with agitation using a magnetic stirrer (at 200 rpm) during 2 h at room temperature (20 °C). Chitosan (CH) solution (1 mg/mL) was prepared in 1% of lactic acid, through the same methodology used for the ALG solution (1 mg/mL). The pH of polyelectrolyte solutions was adjusted with 0.1 mol/L sodium hydroxide (NaOH) or with 0.1 mol/L lactic acid solutions.

2.2.2. Assessment of solutions charge

In LbL deposition the pH of solutions (CH, ALG, 5-ASA and GMP) must be adjusted in order to guarantee a correct electrostatic interaction between the layers and/or components of the capsule, i.e. between PS nanoparticles, 5-ASA, GMP, ALG and CH layers. Likewise, in order to achieve the aforementioned conditions, it is crucial to choose the adequate pH for each solution (e.g. below or above the pK_a and/or p*I*), where the solutions should exhibit opposite surface charges (positive or negative).

2.2.2.1. Chitosan and alginate. Chitosan (CH) and alginate (ALG) are weak polyelectrolytes, meaning that their dissociation degree depends strongly on the solution pH (below or above the pK_a). It was previously reported that when the pH of ALG solution is below or close to the pK_a value (~3.4–4.4), a drastic decrease in the ionization degree of ALG macromolecules is observed leading to a lack of sufficient charge, which would difficult its self-assembly deposition [6]. In the case of CH, it is only soluble in acidic solutions, where the pH value is already distant from its pK_a (6.2–7.0) [2]. Based on this information, the pH values for ALG and CH deposition were fixed at 6.0 and 3.0, respectively. The behaviour of CH and ALG was therefore assessed using these pH values in order to ensure their polycation and polyanion behaviour, respectively.

2.2.2.2. Model bioactive compounds. Glycomacropeptide (GMP) and 5-aminosalycic acid (5-ASA) were chosen as bioactive compound models. GMP solution (1 mg/mL) was prepared dissolving GMP in distilled water with agitation using a magnetic stirrer (at 200 rpm) during 2 h at room temperature (20 °C). 5-ASA solution (1 mg/mL) was prepared in 2% of lactic acid, through the same methodology used for the GMP solution.

Being assembled onto a negative charged polyelectrolyte (ALG), BCs must present a positive surface charge in order to allow electrostatic interactions between layers. This is achieved by choosing the pH value for GMP and 5-ASA solutions, which corresponds to the highest positive zeta potential value. Regarding GMP, it was previously reported [18] that the solution exhibits a positive and maximum zeta potential value at pH = 2, while for 5-ASA, preliminary experiments showed that pH = 2.3 is the most adequate value to obtain those conditions (results not shown). The pH of bioactive compound solutions was adjusted with 0.1 mol/L sodium hydroxide (NaOH) or with 0.1 mol/L lactic acid solutions.

2.2.3. Preparation of chitosan/alginate multilayer nanocapsules

Multilayer nanocapsules have been prepared in aqueous media by LbL deposition of CH and ALG onto PS nanoparticles according to Ye et al. [6], with some modifications:

(1) PS nanoparticles aqueous dispersion (0.5 mg/mL) was used as the template.
(2) First layer was deposited with the addition of 1 mL of CH solution into 0.5 mL of PS nanoparticles solution. The mixture was incubated at room temperature, for 10 min under gentle agitation.
(3) The excess of polyelectrolyte was removed by two repeated cycles of centrifugation (18,625 × *g* for 20 min, MIKRO 120, Hettich, Germany) with consequent removal of supernatant and redispersion in water (pH 7), discarding the unadsorbed CH in the suspension. In addition, sonication was used to improve the dispersion of the capsules in the solution by preventing their fast agglomeration [19,20]. Moreover, sonication also allows exploiting maximum surface area for subsequent polyelectrolyte deposition [21]. For this purpose, sonication (40 kHz, 30 min) was used after the cycles of centrifugation.
(4) The following ALG layer was deposited with 1 mL ALG solution (1 mg/mL) using the same procedure described in steps 2 and 3.

This procedure was repeated until three CH/ALG layers were assembled onto PS nanoparticles (Fig. 1), resulting in PS-$(CH\text{-}ALG)_3$ nanocapsules. All sets of experiments were made at least in duplicate.