Journal of Colloid and Interface Science 404 (2013) 85–90

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Regular Article

Preparation of monodisperse calcium alginate microcapsules via internal gelation in microfluidic-generated double emulsions

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ARTICLE INFO

Article history:
Received 20 March 2013
Accepted 26 April 2013
Available online 7 May 2013

Keywords:
Microcapsules
Calcium alginate
Internal gelation
Microfluidic emulsification
Calcium carbonate nanoparticles

ABSTRACT

Monodisperse hollow and core–shell calcium alginate microcapsules are successfully prepared via internal gelation in microfluidic-generated double emulsions. Microfluidic emulsification is introduced to generate monodisperse oil-in-water-in-oil (O/W/O) double emulsion templates, which contain Na-alginate, $CaCO_3$ nanoparticles, and photoacid generator in the middle aqueous phase, for synthesizing Ca-alginate microcapsules. The internal gelation of the aqueous middle layer of O/W/O double emulsions is induced by crosslinking alginate polymers with Ca^{2+} ions that are released from $CaCO_3$ nanoparticles upon UV exposure of the photoacid generator. The as-prepared hollow and core–shell calcium alginate microcapsules are highly monodisperse and spherical in water. Model proteins Bovine serum albumin (BSA) molecules can be encapsulated into the Ca-alginate microcapsules after the capsule preparation, which demonstrates an alternative route for loading active drugs or chemicals into carriers to avoid the inactivation during the carrier preparation. The proposed technique in this study provides an efficient approach for synthesis of monodisperse hollow or core–shell calcium alginate microcapsules with large cavity or encapsulated lipophilic drugs, chemicals, and nutrients.

1. Introduction

Alginates have been one of the most popular biomaterials because of the convenient sources, nontoxicity, excellent biocompatibility, and biodegradability [1,2]. Alginates are anionic polysaccharides composing of β-D-mannuronic acid and α-L-guluronic acid, and they form hydrogels with multivalent cations such as Ca^{2+}, Ba^{2+}, or Fe^{3+} [3]. The preparation process of alginate microspheres or microcapsules involves two steps, i.e., droplet generation and ionic crosslinking. In traditional droplet generation technique, the sodium alginate solution is directly dripped into $CaCl_2$ solution to form spherical and uniform calcium alginate hydrogel beads, but the resulted beads are usually with large size (millimeter size) due to the limitations of large needle size and large solution viscosity [3]. In order to overcome the issues in the traditional droplet technique, various novel techniques such as electrostatic droplet generation [4,5] and membrane emulsification [6] have been employed to reduce the size of droplets, from which micron-sized alginate microspheres could be prepared via external or internal gelation.

The recently developed microfluidic emulsification technique is able to generate droplets with size ranging from dozens to hundreds of micrometers, which provides possibilities for producing monodisperse calcium alginate microspheres or microcapsules with micron size. To prepare calcium alginate microspheres or microcapsules in microfluids, there are four main methods as follows: (1) external gelation. There are two approaches according to the introduction of a crosslinker. One is that the crosslinker is dissolved in the oil phase (continuous phase) at first and then diffuses from the oil phase to the aqueous phase after the droplets are generated. As the gelation advances inward, a "skin" layer appears on the surface of the alginate droplet, so the surface of the resultant microspheres is not very smooth [7]. The other approach is that the crosslinker is dissolved in the aqueous gelling bath outside the microfluidic device where the microfluidic generated alginate droplets are collected [8–10], or the gelling bath with the crosslinker is introduced in the downstream in the microfluidic device where the droplets gelate in a flowing state [11]. The external gelation is so fast that deformed microspheres with "tails" are often obtained due to the deformation when the droplets transfer from the oil phase to the gelling bath. (2) Droplet coalescence. Sugiura et al. [12] and Liu et al. [13] generated Na-alginate droplets and $CaCl_2$ droplets separately in two independent channels. Then, coalescence of Na-alginate droplets and $CaCl_2$ droplets is initiated in

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0021-9797/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2013.04.044

the synthesizing channel to produce calcium alginate microspheres. The feasibility of this method is quite dependent on the collision possibility of the two kinds of droplets. Another disadvantage of this method is the wide size distribution of the coalesced droplets [14]. (3) In situ mixing. Xu et al. [15] designed a microfluidic device which consists of two crossjunctions and a synthesizing channel for production of calcium alginate microspheres. Na-alginate solution and $CaCl_2$ solution are the two side streams at the first junction which are separated by the deionized water injected from the middle channel. Then, fluid mixture is sheared into droplets by focusing of the oil phase, which gels in the synthesizing channel downstream. One of the drawbacks of this method is the limited mixing efficiency under laminar flow condition. Of course, chaotic mixing can be achieved by winding the synthesizing channel to enhance the mixing efficiency [16]. (4) Internal gelation. So far, there are not many successful applications of internal gelation of calcium alginate microspheres in microfluidic device because of two limitations: one is the sediment and heterogeneous dispersion of the $CaCO_3$ powder [17], and the other is the poor mechanical strength of the resultant calcium alginate microspheres [18].

Most of the attempts to prepare alginate microcapsules using single emulsions as templates usually result in solid microspheres. Although calcium alginate microcapsules with lower crosslinking degree inside can be obtained via external gelation, the inner cavities of those microcapsules are not well-defined, because the precise control of crosslinking process dependent on the gelation time and crosslinker concentration is still difficult. Up to now, only a few researchers have tried to prepare calcium alginate microcapsules in microfluidic device based on double emulsions, but sometimes the shapes of the microcapsules were uncontrollable and non-spherical because of the external gelation [11].

In this paper, internal gelation is combined with microfluidic emulsification to prepare micron-sized monodisperse calcium alginate microcapsules. Oil-in-water-in-oil (O/W/O) double emulsions containing Na-alginate in the middle aqueous layer are generated using a capillary microfluidic device and serve as templates for synthesis of calcium alginate microcapsules, as shown in Fig. 1. The aqueous phase of the double emulsions contains sodium alginate, $CaCO_3$ nanoparticles, and a photoacid generator. Upon UV irradiation, the photoacid generator releases protons, as a result the pH value decreases. Therefore, calcium ions are released from calcium carbonate and crosslink the alginate polymers [19]. The as-prepared calcium alginate microcapsules are highly monodisperse and spherical in water and with well-defined cavity. Furthermore, oily substances and a model protein Bovine serum albumin (BSA) molecules can be encapsulated inside the calcium alginate microcapsules with the proposed process.

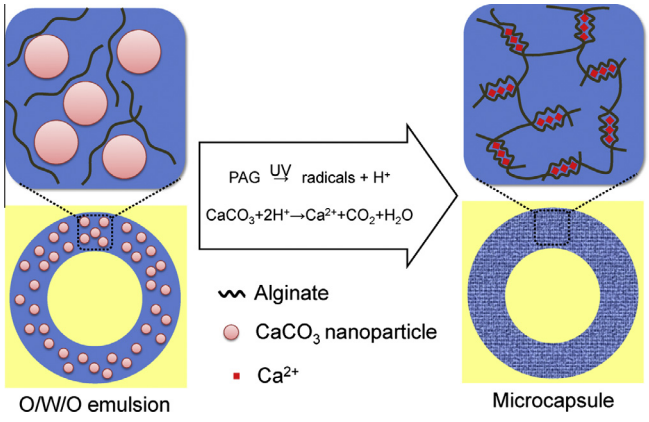

Fig. 1. Schematic illustration of fabrication of calcium alginate microcapsule via internal gelation in O/W/O double emulsion. "PAG" stands for diphenyliodonium nitrate.

2. Materials and methods

2.1. Materials

Sodium alginate (C.P.) was purchased from Kermel Chemical Reagent (Tianjin, China). $CaCO_3$ nanoparticles with an average size of 50 nm were provided by Ruicheng Huana Nanomaterials (Shanxi, China). Diphenyliodonium nitrate (PAG) was purchased from TCI (Shanghai, China). Soybean oil was provided by Kerry Oils & Grains (China). Polyglycerol polyricinoleate (PGPR) was purchased from Danisco (Denmark). Benzyl benzoate was obtained from Sinopharm Chemical Reagent (Shanghai, China). Lumogen® F Red 300 (LR300) was provided by BASF. Bovine serum albumin (BSA) was obtained from Amresco. Pluronic F127 (F127) and fluorescein isothiocyanate (FITC) were obtained from Sigma–Aldrich. Isopropanol was purchased from Kelong Chemical Reagent (Chengdu, China). All the chemicals are reagent grade. Deionized water from Millipore Milli-Q water purification system was used throughout the experiments.

2.2. Fabrication of microfluidic device

The capillary microfluidic device was fabricated by assembling glass capillary tubes on glass slides [11,20]. The outer diameters of all the cylindrical capillary tubes were 1.0 mm. The square capillary tubes had an inner dimension of 1.0 mm. The inner diameters of the injection tube, the transition tube, and the collection tube were 550, 250, and 550 μm, respectively. A micropuller (Narishige) was used to taper the end of cylindrical capillaries, and the orifice dimensions of tapered ends were adjusted by a microforge (Narishige). The inner diameters of the tapered end of the injection and transition tubes were 60 and 200 μm, respectively. All cylindrical capillary tubes were coaxially aligned within the square capillary tubes by matching the outer diameters of the cylindrical tubes to the inner dimensions of the square ones.

2.3. Preparation of monodisperse calcium alginate microcapsules

A mixture of soybean oil and benzyl benzoate (1:1 v/v) was used as inner oil phase. LR300 was added into inner oil phase for preparation of core–shell calcium alginate microcapsules. The middle aqueous phase contained 1 wt% F127, 1.5 g/L $CaCO_3$ nanoparticles, 2 wt% sodium alginate, and 30 mmol/L PAG. The outer oil phase was soybean oil with 8% (w/v) PGPR.

To prepare monodisperse O/W/O double emulsions as templates, inner oil phase, middle water phase, and outer oil phase solutions were separately pumped into the injection tube, the transition tube, and the collection tube through polyethylene tubing attached to disposable syringes. The fluid flows were driven by syringe pumps (LSP01-1A, Baoding Longer Precision Pumps). Based on the coaxial co-flow geometry, monodisperse O/W single emulsions were generated in the transition tube and monodisperse O/W/O double emulsions were generated in the collection tube. The flow rates of the inner, middle, and outer fluids were $Q_1 = 300\ \mu L\ h^{-1}$, $Q_2 = 400\ \mu L\ h^{-1}$, and $Q_3 = 4000\ \mu L\ h^{-1}$, respectively. The obtained O/W/O double emulsions were collected in a petri dish and were subjected to UV irradiation (250 W, 250–450 nm) in ice bath for 30 min.

To obtain hollow microcapsules, the inner and outer oil phases of the products were washed with isopropanol, and the microcapsules were finally dispersed in water. To obtain core–shell microcapsules, only the supernatant oil was removed. Then, the