Journal of Colloid and Interface Science 514 (2018) 156–164

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

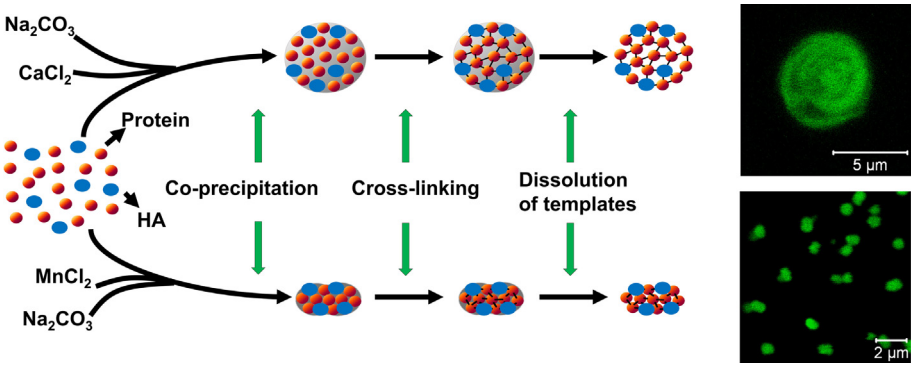
journal homepage: www.elsevier.com/locate/jcis

Structure and properties of hybrid biopolymer particles fabricated by co-precipitation cross-linking dissolution procedure

Yu Xiong *, Radostina Georgieva, Axel Steffen, Kathrin Smuda, Hans Bäumler *

Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Transfusion Medicine, Charitéplatz 1, 10117 Berlin, Germany

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 September 2017
Revised 6 December 2017
Accepted 11 December 2017
Available online 12 December 2017

Keywords:
Co-precipitation
Composite materials
Microstructures
Calcium carbonate
Manganese carbonate
Hyaluronic acid

ABSTRACT

The Co-precipitation Crosslinking Dissolution technique (CCD-technique) allows a few-steps fabrication of particles composed of different biopolymers and bioactive agents under mild conditions. Morphology and properties of the fabricated biopolymer particles depend on the fabrication conditions, the nature of the biopolymers and additives, but also on the choice of the inorganic templates for co-precipitation. Here, we investigate the influence of an acidic biopolymer, hyaluronic acid (HA), on the formation of particles from bovine hemoglobin and bovine serum albumin applying co-precipitation with $CaCO_3$ and $MnCO_3$. $CaCO_3$ templated biopolymer particles are almost spherical with particle size from 2 to 20 µm and protein entrapment efficiency from 13 to 77%. Presence of HA causes significant structural changes of the particles and decreasing protein entrapment efficiency. In contrast, $MnCO_3$ templated particles exhibit uniform peanut shape and submicron size with remarkably high protein entrapment efficiency of nearly 100%. Addition of HA has no influence on the protein entrapment efficiency or on morphology and size of the particles. These effects can be attributed to the strong interaction of Mn^{2+} with proteins and much weaker interaction with HA. Therefore, entrapment efficiency, size and structure of biopolymer particles can be optimized by varying the mineral templates and additives.

1. Introduction

Colloidal systems offer numerous biological, medical and pharmaceutical applications as biosensors, bioreactors, diagnostic tools and drug delivery systems [1–4]. In particular, biopolymer based

* Corresponding authors.
E-mail addresses: yu.xiong@charite.de (Y. Xiong), hans.baeumler@charite.de (H. Bäumler).

https://doi.org/10.1016/j.jcis.2017.12.030
0021-9797/

particles have attracted considerable attention for medical applications. For the preparation of protein based particles or capsules, several methods exist including desolvation technique, double emulsion technique, spray drying, liposome encapsulation, or layer-by-layer (LBL) polyelectrolyte encapsulation [5–11].

Inspired by studies on biomineralization processes inorganic calcium carbonate ($CaCO_3$) and manganese carbonate ($MnCO_3$) particles have been intensively investigated as entrapment tools for biomolecules. These inorganic particles are easily synthetized by simple procedures at mild fabrication conditions usually via precipitation reactions by mixing two salt solutions [11–16] and have been observed to have a high absorption capacity for polymers and biomacromolecules [3,11,12,16]. Several applications have been reported in diverse scientific publications, for example as templates for fabrication of polyelectrolyte multilayer capsules [3,11,12].

In mineralized tissues, such as bone, cartilage, etc. calcium phosphate or carbonate crystalline or amorphous structures are formed through interactions with biomacromolecules. In fact, precipitation of $CaCO_3$ in the presence of biopolymers has become interesting as biopolymers can be captured more effectively than by simple adsorption onto preformed $CaCO_3$ particles [17]. Such biopolymer-$CaCO_3$ hybrid particles provide a suitable platform for fabrication of LbL-microcapsules containing encapsulated proteins [17–19].

A more facile and effective method for the fabrication of biopolymer particles [20], which is also based on co-precipitation of carbonates with biopolymers was developed in our department [21–24]. The co-precipitated biopolymers form insoluble biopolymer-carbonate hybrid particles with the metal carbonates and are intermolecularly cross-linked. Subsequently, the metal carbonate templates are dissolved to yield high density pure biopolymer particles. We call this method CCD-technique according to the three key steps, namely Co-precipitation, Cross-linking (of the biopolymers) and Dissolution of the templates [25].

Previously we reported the potential biomedical application of hemoglobin particles prepared by the CCD technique using $CaCO_3$ and $MnCO_3$ templates as artificial oxygen carriers [23,24].

The remarkable differences between the hemoglobin particles fabricated using $CaCO_3$ and $MnCO_3$ in terms of particle morphology, size and in particular of the protein entrapment efficiency attracted our attention. While the protein capture mechanism during protein/$CaCO_3$ co-precipitation is well documented [17], the mechanism of the high protein entrapment efficiency by protein co-precipitation with $MnCO_3$ is less investigated. The morphology and the size of carbonate microparticles strongly depend on the precipitation conditions and on the presence of additives [26–31]. The crystallization process of $CaCO_3$ is influenced by the presence of some polymers, especially acidic polymers [27–29,32,33]. However, the influence of such acidic polymers mixed with proteins on the formation of particles via the CCD-technique is unknown. Since multifunctional nano- and microparticles for medical applications or diagnostic purposes contain more than one compound and can be composed by different polymers, it is important to investigate how the different polymers influence the formation and structure of particles.

In the current study, we applied the CCD technique to prepare particles made of hemoglobin or bovine serum albumin using $CaCO_3$ or $MnCO_3$. Hyaluronic acid, an acidic biopolymer [34], was applied as an additive to the proteins to investigate its influences on the morphology of the particles and on the protein entrapment efficiency. Hyaluronic acid was used in the past for the fabrication of Layer by Layer polyelectrolyte microcapsules templated on $CaCO_3$ [12,35]. Recently, a paper about hyaluronic acid templated $CaCO_3$ using another technique ($ScCO_2$ process) without crosslinking and dissolution was published by Ramalapa et al. [36]. To our best knowledge, there are no other reports about the hyaluronic acid mediated formation of $CaCO_3$, $MnCO_3$ or other types of mineral particles.

2. Materials and methods

2.1. Materials

Hemoglobin (Hb) was extracted from bovine red blood cells by hypotonic hemolysis [24,37]. Bovine serum albumin (BSA), fluorescein isothiocyanate (FITC)-BSA, hyaluronic acid sodium salt (HA) with the molecular weight of approximately 1.5–1.8 $\times 10^6$ Dalton, glutaraldehyde (GA), divinyl sulfone (DVS), rhodamine 6G, calcium chloride ($CaCl_2$), manganese chloride ($MnCl_2$) tetrahydrate, sodium carbonate (Na_2CO_3), phosphate buffered saline (PBS) pH 7.4, ethylenediaminetetraacetic acid disodium salt (EDTA), sodium hydroxide (NaOH), glycine and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich; Ampuwa® (aqua ad injectable) and sterile 0.9% NaCl solution were purchased from Fresenius Kabi Deutschland GmbH.

2.2. Preparation of particles

Pure protein particles were fabricated as previously described [21,23,24]. Briefly, equal volumes of 0.25 M Na_2CO_3 and 0.25 M $CaCl_2$ or 0.25 M $MnCl_2$ containing 2.5–15 mg mL^{-1} Hb or BSA were mixed in a beaker under stirring (stirring speed from 200 to 600 RPM) at room temperature. For fluorescence labelling, FITC-BSA (weight ratio to Hb or BSA = 1:50) was added to $CaCl_2$ or $MnCl_2$. The obtained protein-metal carbonate hybrid particles were separated by centrifugation and washed three times with Ampuwa®. The particles were suspended in GA solution (0.01–0.1%) and incubated at room temperature for 1 h, followed by centrifugation and quenching of remaining GA with glycine. The $CaCO_3$ or $MnCO_3$ templates were dissolved by adding EDTA solution (0.2 M, pH 7.4) and the particles were treated with $NaBH_4$. Finally, the resulting particles were centrifuged, washed three times and resuspended in Ampuwa® until further use.

For preparation of protein-HA particles, HA (weight ratio to protein = 1:10 or 1:20) was added to the $CaCl_2$ or $MnCl_2$ solution and the cross-linking step was performed with 0.1% GA containing 50 mM DVS at room temperature. For pure HA particles, co-precipitation was carried out under fast stirring by mixing equal volumes of 0.25 M Na_2CO_3 containing 2.5 mg mL^{-1} HA and 0.25 M $CaCl_2$ at 600 RPM on a magnetic stirrer. The cross-linking step was performed with 100 mM DVS in 0.1 M NaOH over 18 h at room temperature.

2.3. Protein entrapment efficiency (EE)

EE of $MnCO_3$ or $CaCO_3$ particles was determined as the difference between the total protein amount applied (PAt) and the protein amount determined in the supernatant (PAf) after co-precipitation and after each washing step. The EE% was calculated according to the following equation EE% = (PAt − PAf) × 100%/PAt. The measurements of Hb concentration were performed with a microplate reader (PowerWave 340, BioTek Instruments GmbH) at 415 nm and the measurements of BSA concentration were performed with UV–VIS-spectrophotometer (Hitachi U2800, Hitachi High-Technologies Corporation) at 280 nm.

2.4. Confocal laser scanning microscopy (CLSM)

CLSM images were taken with a confocal microscope LSM 510 Meta (Carl Zeiss MicroImaging GmbH, Jena, Germany) equipped