Sensors and Actuators B 256 (2018) 217–225

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Research paper

Construction of biconcave hemoglobin-based microcapsules and electrochemical evaluation for its ability of oxygen carry

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ARTICLE INFO

Article history:
Received 2 June 2017
Received in revised form 3 September 2017
Accepted 25 September 2017
Available online 27 September 2017

Keywords:
Hemoglobin-based oxygen carriers
$Ca(OH)_2$
Oxygen-carrying capability
Co-precipitation
Electrochemical technique

ABSTRACT

Hemoglobin (Hb)-based oxygen carriers (HBOCs) have been investigated intensively. Enhancing the loading content of Hb in a red-blood-cell (RBC) like structure and seeking effective method to evaluate their oxygen-carrying capability have been the significant challenges. Here, we present unique biconcave Hb-$Ca(OH)_2$ microcapsules of around 6.5 μm with high oxygen affinity using a facile and controllable method. Dextran introduced in the co-precipitation process can not only adjust the morphology of $Ca(OH)_2$ particles but also improve the biocompatibility of the obtained microcapsules. The Hb entrapment efficiency in the microcapsules corresponded to 68% of the Hb content in one native RBC. The dynamic process of carrying-releasing oxygen of Hb-$Ca(OH)_2$ microcapsules was investigated using electrochemical techniques. Hb in microcapsules on the surface of electrode maintained its naturally biological activity and exhibited a high ability to carry oxygen reversibly. The oxygen releasing of oxyHb-$Ca(OH)_2$ microcapsules can be retained during at least 2400 s. The results demonstrate that Hb-$Ca(OH)_2$ microcapsules with highly loaded content possessed excellent function of oxygen-binding and releasing, which make it promising for applications in artificial oxygen carriers and other biomedical fields.

1. Introduction

For blood transfusion in various kinds of emergency, the risk of infection from blood and blood products, and requirement of right type matching prior to transfusion have motivated development of "universal" blood substitutes and red blood cell (RBC) replacements [1]. Hemoglobin (Hb) is a vital protein in RBC, and is responsible for storage and transport of oxygen and other gaseous ligands [2]. Due to the unique carrying/delivering oxygen capacity, Hb has become a potential candidate as RBC substitute for use in transfusion medicine [3]. However, cell-free Hb is not suitable for an ideal oxygen donor because it produces severe problems, including short circulation time, potential side effect, and poor stability [4]. Aiming at stabilizing Hb in a tetrameric form or in a polymeric form, diverse modifications of Hb based on intra- and intermolecular cross-linking or encapsulation have been investigated to find suitable oxygen carriers during the last 30 years [5–7]. Various types of Hb-based oxygen carriers (HBOCs) have been developed as blood substitutes for the clinical and preclinical application, which can overcome some limitations existing in modern transfusion, such as limited availability of blood donors, difficulty in blood type matching, hemolytic transfusion reactions, etc [8,9].

Recently, a new trend has emerged in the HBOCs field, which focuses on the development of nanobiotechnology for encapsulation Hb into carriers and has shown promise in research and clinical trials [10]. They are different kinds of encapsulated HBOCs in nano- or microscale, that Hb molecules are incorporated with different materials and designed to mimic the features of RBCs. For instance, Jia et al. fabricated $(Hb/DHP)_6$ microcapsules by covalent layer-by-layer assembly and demonstrated that the microcapsules possessed the essential function of an oxygen carrier [11]. Xiong et al. presented Hb particles of around 700 nm based on $MnCO_3$ particles with co-precipitation of Hb, which possess high oxygen affinity and can avoid vasoconstriction of small blood vessels [12]. Nonetheless, the shape of these Hb-based oxygen carriers is usually spherical and strikingly different in comparison with RBCs. In fact, RBCs with their unique biconcave discoidal morphology represent a remarkable example of structure enabling the implementation of sophisticated biological functionalities [13]. Therefore, it has remained challenging to design and synthesize RBC-like oxygen-carrying particles with a biconcave morphology, which provides a favorable surface-area-to-volume ratio and allows them to perform important and complex biofunctions.

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https://doi.org/10.1016/j.snb.2017.09.166
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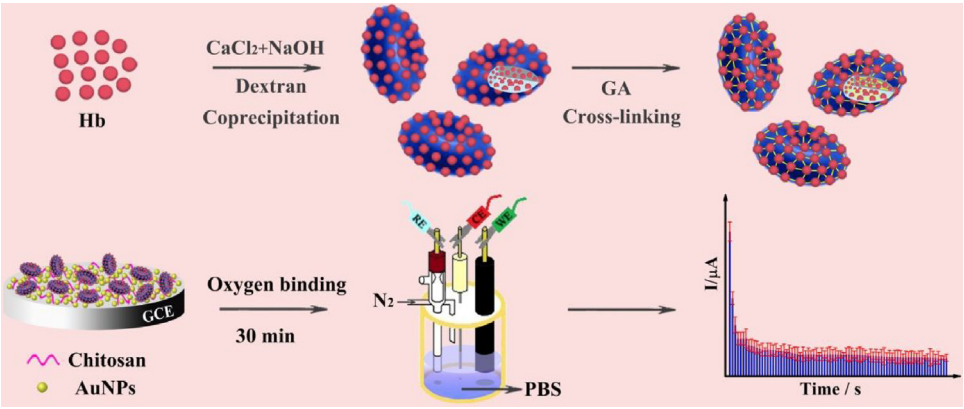

Scheme 1. Schematic illustration to show the fabrication process of Hb-$Ca(OH)_2$ microcapsules and electrochemical study of their oxygen releasing.

The most important issue for artificial oxygen carriers is related to their primary function of transporting oxygen. Usually, the oxygen-carrying ability was investigated by the venous blood gas analysis and other clinical methods [14,15], which are not easy ways to monitor the oxygen-carrying capacity quantitatively and dynamically. Electrochemical methods have the advantages of high sensitivity, selectivity, low cost and simplicity [16], especially in the electrochemical study of oxygen sensors based on redox proteins [17,18]. Studies on the electrochemistry of Hb are an essential research field for understanding the property and the ability of carrying oxygen of Hb-based oxygen carriers.

In this work, as shown in Scheme 1, we firstly fabricated biconcave discoidal Hb-$Ca(OH)_2$ microcapsules that imitating the size and shape of RBCs by co-precipitation of Hb and $Ca(OH)_2$. The introduction of dextran sulfate (DS) during the reaction process resulted in $Ca(OH)_2$ particles of a RBC-like morphology. Dextran is a water-soluble polysaccharide and one of the most common coatings utilized in various clinical trials. Due to its excellent biocompatibility, long blood circulation time and high bio-affinity [19], it can be not only used as an antithrombotic to reduce blood viscosity, but also designed as various polymeric nanocarriers for controlled drug delivery [20]. It is reported that dextran can be adsorbed on the preformed particles and subsequent influence the particle deposition [21]. Therefore, the introduction of dextran in this work can not only increase the biocompatibility and biodegradability of the Hb-$Ca(OH)_2$ microcapsules but also generate the Hb-based microcapsules with RBC-like morphology.

The morphological and physicochemical properties of the synthesized Hb-$Ca(OH)_2$ microcapsules were characterized by spectral analysis. The oxygen-carrying and releasing capability of the Hb-$Ca(OH)_2$ microcapsules was then evaluated using electrochemical techniques. Results demonstrate that biconcave RBC-like microcapsules prepared by this method can not only keep the bioactivity of Hb assembled in the microcapsules, but also have the ability to carry and release oxygen reversibly that is similar to RBCs, which endows these microcapsules with great potential to function as oxygen carriers.

2. Experimental

2.1. Reagents and apparatus

Bovine Hb, glutaraldehyde (GA) and chitosan (CS, ≥90% deacetylation) were obtained from Sinopharm Chemical Reagent Co., Ltd. Dextran sulfate (DS) (Mw = 500 kDa), chloroauric acid ($HAuCl_4{\cdot}4H_2O$, 99.999%, Au% >48%), sodium citrate, sodium hydroxide (NaOH) and calcium chloride ($CaCl_2$) were purchased from Sigma (Shanghai, China) and used as received. Phosphate buffer solutions (PBS, 0.1 M) with different pH values were prepared by mixing Na_2HPO_4 and NaH_2PO_4 solutions and using 0.1 M HCl or NaOH solution to adjust the pH. All chemicals were of analytical grade and used without further purification. All the aqueous solutions were prepared with deionized water.

The morphology of Hb-$Ca(OH)_2$ microcapsules was characterized by the scanning electron microscopy (SEM) with a JSM-6510 microscope. X-ray diffractions (XRD) were obtained by X'Pert-Pro MPD X-ray diffractometer (Panalytical, Holland) using Cu Kα radiation of with 40 kV and 40 mA. X-ray photoelectron spectroscopy (XPS, Escalab 250Xi, Thermo Scientific, USA) was used to analyze the elements of Hb-$Ca(OH)_2$. UV–vis spectra were recorded using an UV-2450 spectrophotometer. Fourier transform-infrared (FT-IR) spectra were obtained with an AVATAR-370 (Nicolet) spectrometer. Confocal laser scanning microscopy (CLSM) micrographs were taken with a Leica TCS-SP2 system equipped with 100× oil-immersion objective and a numerical aperture of 1.4. Electrochemical measurements were performed with a CHI 660D electrochemical workstation (Shanghai CH Instrument Co. Ltd., China) using the modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode (WE). A saturated calomel electrode (SCE) and a platinum wire electrode served as the reference electrode (RE) and the counter electrode (CE), respectively.

2.2. Preparation of Hb-$Ca(OH)_2$ microcapsules

The $Ca(OH)_2$ microparticles were fabricated according to the literature [22]. To prepare the biconcave discoidal Hb-$Ca(OH)_2$ microcapsules, DS (0.5 g) was dissolved in $CaCl_2$ solution (50 mL, 0.1 M), into which Hb solution (50 mL, 5 mg mL^{-1}) was rapidly poured. Then NaOH solution (40 mL, 0.5 M) was added quickly to the above mixture under vigorous stirring at room temperature for 2 min. This system was allowed to stand for 20 min. The precipitated Hb-$Ca(OH)_2$ microcapsules were collected by centrifugation. Then the Hb-$Ca(OH)_2$ microcapsules were suspended in GA (0.025%, 20 mL) for 2 h to prevent the dissociation of Hb [23]. After centrifuged and washed with deionized water and ethanol thrice, the Hb-$Ca(OH)_2$ microcapsules were collected and stored at 4 °C. For comparison, the biconcave discoidal $Ca(OH)_2$ microcapsules were fabricated using the similar method without Hb. Pure $Ca(OH)_2$ particles were synthesized by rapidly pouring NaOH solution (20 mL, 0.5 M) into $CaCl_2$ (50 mL, 0.05 M) solution with constant stirring at room temperature.

Hb amount in Hb-$Ca(OH)_2$ microcapsules was determined as the difference between the total Hb amount applied (Hb_t) and the Hb amount determined in the supernatant (Hb_f) after co-precipitation and after each washing step. The encapsulation efficiency (EE%) was calculated according to the following equation