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Construction of electrochemical avenue for evaluating oxygen-carrying performance of a microsphere-based oxygen carrier with bovine serum albumin protection layer

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ABSTRACT

The production of oxygen carriers is currently a topic of paramount interest in nanotechnology. Particularly, there is a focus in the development of microparticles with round and uniform shapes to further expand their potentialities. Here, we prepare homogenous microspheres with diameters of ~3 μm using porous calcium carbonate spheres as the template and glutaraldehyde as the cross-linking agent. Also, we present for the first time the use of electrochemical avenue to evaluate the oxygen-carrying ability of these as-prepared samples. Our results demonstrate successful construction of prospective microspheres which perform extraordinary oxygen-carrying capability with protection of bovine serum albumin layer on the surface.

(See Schemes 1 and 2.)

1. Introduction

During the past decades, with the aim to address ongoing clinical needs, researchers have expended considerable efforts in developing suitable technologies for the production of oxygen carriers, especially the oxygen-containing microparticles [1–4]. Renato et al. concluded that the shape of microparticles plays more important role in the fields of drug delivery and tissue engineering, and round or spherical microspheres might be the optimal shape in most related events [5]. When compared to those techniques [6,7], protein encapsulation technology has been one of the optimized avenues for the production of microspheres [8,9]. Therefore, driven by the willingness to better exploit the advantages related use of microspheres-based oxygen carriers, preparation of round or spherical oxygen-carrying microparticles with protein encapsulation process can be one of the prospective methods.

Up to date, two main proteins have been reported for the production of oxygen carriers. On the one hand, hemoglobin, consisting of four polypeptide chains with one heme group at each chain, possesses the ability to deliver and release oxygen in red blood cells (RBCs) [10]. On the other hand, serum albumin, as the most abundant protein in blood plasma, is a common stabilizing element for pharmaceutical and biological use [11]. Also, the addition of serum albumin can dramatically improve its dispersity and prevent agglomeration [12]. As a result, combination of hemoglobin and serum albumin might be preferential for construction of round and homogenous microspheres as oxygen carriers.

One of the central features that determine the performance of microsphere-based oxygen carriers is their oxygen-carrying capability [6]. As a consequence, finding new ways to evaluate the property of microspheres is of large importance to widen the potentialities of oxygen carriers. Electrochemical method has the advantages of high sensitivity, selectivity and simplicity [13,14], especially in the study of the redox protein based electrochemical oxygen biosensor [15,16]. Thus, the development of electrochemistry leading to the fabrication of electrochemical oxygen biosensors containing hemoglobin and serum albumin might provide an exciting novel approach to gain a new avenue to estimate the function of oxygen carriers.

Here, we present a preparation process of uniform hemoglobin-bovine serum albumin (Hb-BSA) microspheres using $CaCO_3$ as the templates and glutaraldehyde as the cross-linking agent [17,18]. Furthermore, electrochemical approach for the first time demonstrates the capability of the obtained samples. Interestingly, the Hb-BSA microspheres modified electrode exhibits enhanced capacity to store and transport

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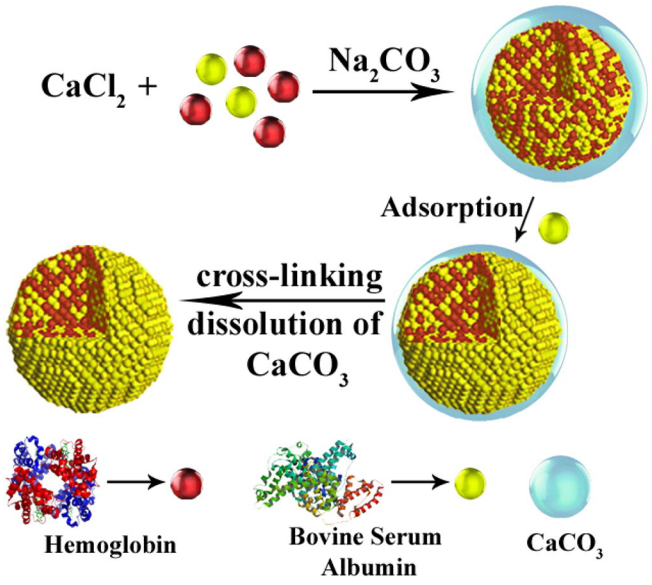


Scheme 1. Schematic illustration of the assembly process of Hb-BSA microspheres.

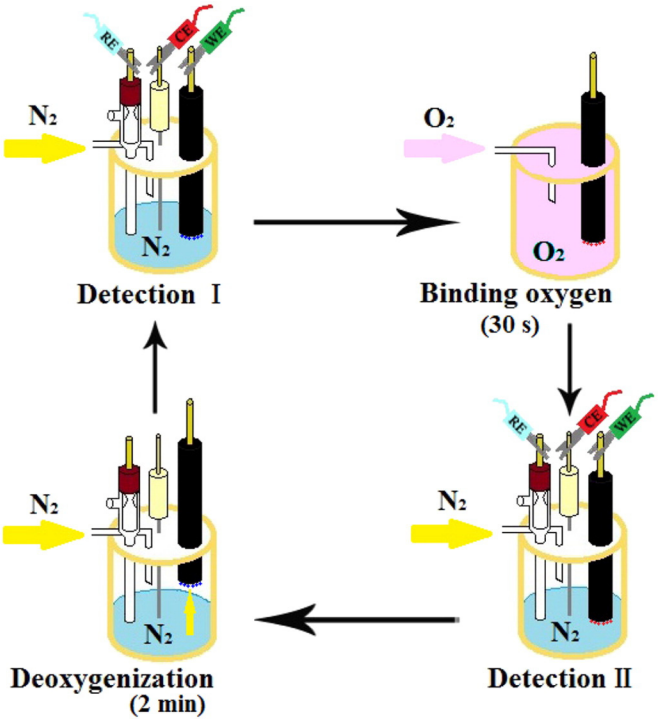
oxygen when compares with pure Hb-coated microspheres, indicating that BSA plays an important role in enhancing the oxygen-carrying capacity. In summary, the utilization of electrochemistry for the study of our prepared Hb-BSA microspheres might be a novel effective approach for further application of oxygen carriers in electrochemical field.

2. Materials and Methods

2.1. Apparatus and Reagents

Bovine hemoglobin, ethylenediamine tetraacetic acid disodium salt (Na_2EDTA), L-ascorbic acid and sodium dithionite ($Na_2S_2O_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA) was obtained from Sangon Biotech Company. Glutaraldehyde (25% water solution, GA) was obtained from Shanghai Ling Feng Chemical Reagent Co., Ltd. Phosphate buffer solution (PBS) was prepared by mixing 0.10 M Na_2HPO_4, NaH_2PO_4 and NaCl. All chemicals were of analytical grade and used without further purification. Double-distilled water was used in all the experiments.

Electrochemical measurements were performed with a CHI 660D electrochemical workstation using the modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode, a platinum wire as the counter, and a saturated calomel electrode (SCE) as reference electrode. The morphology and the element of Hb-BSA microspheres were characterized by the scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) with a JSM-6510 microscope. The chemical structure of Hb-BSA microspheres was characterized using Nicolet Avatar-370 FT-IR spectrometry. The UV-vis spectra were recorded on an UV-2450 spectrophotometer.



Scheme 2. The technological process for evaluating oxygen-carrying ability of Hb-BSA microspheres and Hb-coated microspheres modified electrodes.

2.2. Preparation of Hb-BSA Microspheres

Briefly, 0.25 M $CaCl_2$ solution containing 10 mg mL^{-1} hemoglobin and 1 mg mL^{-1} BSA were sonicated at 4 °C for 30 min. Then the equal volume and concentration of Na_2CO_3 solution was rapidly poured into the above mixture. After 2 min, BSA (weight ratio to Hb 1:2) was added, and the suspension was sonicated for 5 min to form the BSA protection layer on the surface of the particles. The precipitation was collected and washed three times with sterile 0.9% NaCl solution by centrifugation. After that, the obtained microspheres were suspended in 0.01% GA in pH 7.0 PBS for 2 h, in order to build imine linkage between aldehyde groups in GA and amino groups in Hb, followed by centrifugation and washing with sterile 0.9% NaCl solution. The $CaCO_3$ template was removed by 0.20 M pH 7.0 Na_2EDTA solution. The Hb-coated microspheres were fabricated by the same method except for adding BSA solution (See Scheme 1).

2.3. The Study of Oxygen-Carrying Capacity

The oxygen-carrying capacity of the Hb-BSA microspheres and Hb-coated microspheres is investigated by the response currents to oxygen based on the electrochemical methods (Scheme 2). The modified electrodes of Hb-BSA microspheres and Hb-coated microspheres were treated in saturated oxygen device for 30 s to bind oxygen, and moved into oxygen-free device (saturated nitrogen) for differential pulse voltammetry (DPV) detection. Then, the electrodes were put in saturated nitrogen for 120 s to release oxygen and detected by DPV. This process was repeated 5 times. For better comparison, all the DPV peak currents were normalized (normalized ratio = DPV peak currents of saturation oxygen or nitrogen treatment/DPV peak currents of initial saturation nitrogen treatment).

3. Results and Discussion

3.1. Characterization of Hb-BSA Microspheres

SEM is often utilized to investigate the morphology of microspheres in different magnifications. The low magnification SEM image (Fig. 1A) shows that Hb-BSA microspheres are well-dispersed with spherical shapes and quite uniform with diameter of ~3 μm. Then, a closer magnification SEM image displays some flaky crystals predicted as BSA attached on the surface of Hb-BSA microspheres (Fig. 1B), which is consistent with the SEM images of BSA-PMMA nanoparticles [19]. Our results also confirm that BSA rather than Hb is likely to be preferentially located on the surface of the microspheres in previous report [20]. By comparison, Hb-BSA microspheres (Fig. 1A and B) demonstrate better dispersity than Hb-coated microspheres (Fig. 1C) due to the BSA-rich surface. Moreover, the EDS data indicates that $CaCO_3$

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