



# Construction of a biocompatible system of hemoglobin based on AuNPs-carbon aerogel and ionic liquid for amperometric biosensor



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## ABSTRACT

Carbon aerogel (CA) composite has potential application in biosensor design. In this article, AuNPs-carbon aerogel (Au-CA) was prepared by ethylene glycol (EG) reducing method, and its morphology was characterized by scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS). Furthermore, a novel biocompatible platform based on Au-CA and ionic liquid (IL) was applied to the immobilization of hemoglobin (Hb) for fabricating amperometric biosensor. Results of spectroscopic and electrochemical examinations indicated that the platform could provide more sites and channels for the immobilization of Hb, aiding in the prevention of the denaturation and leakage of Hb, and enhancing the electron conductivity. In addition, the electrocatalytic property of the biosensor was investigated using hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and nitrite ( $\text{NO}_2^-$ ) as model compounds. The corresponding results revealed the biosensor has fast responses (within 7 s), good dynamic response ranges and low detection limits ( $2.0 \mu\text{M}$  for  $\text{H}_2\text{O}_2$  and  $1.3 \mu\text{M}$  for  $\text{NO}_2^-$ ).

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## 1. Introduction

Amperometric biosensors based upon nanomaterials have stimulated great interest recently because of their unique chemical and physical properties [1,2]. One of the challenges in developing enzymatic biosensors is the immobilization of enzymes on the electrode in high enough quantity to achieve sufficient signal-to-noise (S/N). To date, many kinds of nanomaterials, such as gold nanoparticles (AuNPs) [3], graphene [4], metallic oxides [5] and quantum dots [6] have been used widely in the fabricating biosensors for medical analysis, environmental monitoring and biological molecule detection. Especially, AuNPs with more negative surface charge and large surface areas can adsorb redox proteins or enzymes easily as well as accelerate the electron transfer between immobilized protein and electrode [7,8].

Carbon aerogel (CA), a three-dimensional nano-network structure of primary carbon particles, has attracted considerable attention for both experimental and theoretical scientific communities in recent years [9,10]. Compared with other carbon materials, CA possesses good electrical conductivity, high surface area,

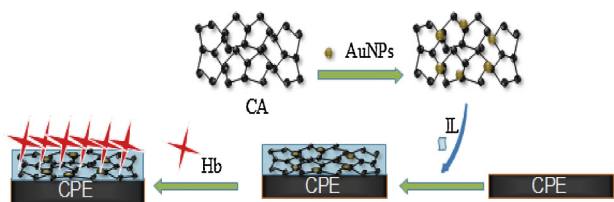
continuous open porosity and biocompatibility. Currently, it is considered as a kind of promising material for electric double layer of supercapacitors, catalyst supports and adsorbents [11–13]. For example, CA, as a support for metal nanoparticles such as Fe-CA, Pt-CA, Pd-CA, exhibits a good catalytic activity [14–16]. In addition, Tang et al. [17] prepared a modified electrode based on Ag-CA nanocomposite, which showed excellent electrocatalytic ability toward halide ion. Our research group reported that three different inorganic/organic doped CA materials (Ni-CA, Pd-CA as well as Ppy-CA) were, respectively, mixed with ionic liquid (IL) to form three stable films for the immobilization of myoglobin (Mb) and the application in  $\text{H}_2\text{O}_2$  detection [18]. The result showed excellent electrocatalytic activity toward the reduction of  $\text{H}_2\text{O}_2$ . The study above encouraged us to pursue the inquiries with new CA-based materials. Nevertheless, to our knowledge, there was no relative report in literatures for the preparation of Au-CA and its application in biosensors. From the view of structure of CA, the networks may provide an attractive scaffold for multilayer enzyme immobilization at an electrode because the network exhibits inertness, rigidity, and negligible swelling in aqueous solution, while still preserve the native structure of the encapsulated enzymes. Therefore, AuNPs-carbon aerogel (Au-CA) would have potential application in biosensor design.

In this work, we first designed the preparation of Au-CA nanocomposite by EG reducing method. Moreover, IL 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid ([BMIm]BF<sub>4</sub>)

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**Scheme 1.** Schematic illustration for preparation of Au-CA and Au-CA/IL/Hb-CPE.

[19] was chosen to disperse Au-CA and to promote the electron transfer of redox proteins. The structure of Hb entrapped in Au-CA/IL composite film was evaluated by UV–vis and FT-IR, while the electrochemical behavior of Hb was investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). In addition, two simple and novel biosensors based on Au-CA/IL were fabricated, and the catalytic ability to  $\text{H}_2\text{O}_2$  and  $\text{NO}_2^-$  was studied, respectively.

## 2. Materials and methods

### 2.1. Reagents

Hemoglobin (Hb, MW 64,500) and  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  were purchased from Sigma Chemical Co.  $[\text{BMIm}]\text{BF}_4$  IL (99%) was purchased from Hangzhou Chemer Chemical Limited Company. High purity graphite powder was obtained from China National Medicine Corporation.  $\text{K}_3\text{Fe}(\text{CN})_6$ ,  $\text{K}_4\text{Fe}(\text{CN})_6$ , KCl,  $\text{K}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{H}_3\text{PO}_4$ , KOH,  $\text{Na}_2\text{SO}_4$ ,  $\text{NaNO}_2$ ,  $\text{HOCH}_2\text{CH}_2\text{OH}$ ,  $\text{CH}_3\text{COCH}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{C}_6\text{H}_6\text{O}_2$ , HCHO (40%),  $\text{H}_2\text{O}_2$  (30%) and liquid paraffin were obtained from Xi'an Chemical Reagent Corporation. All the chemicals were of analytical reagent grade.

0.1 M phosphate buffer solutions (PBS) with different pH values were prepared by mixing the stock standard solutions of  $\text{K}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  and adjusting the pH with 0.1 M  $\text{H}_3\text{PO}_4$  and 0.1 M NaOH. All solutions were made up with twice-distilled water.

### 2.2. Apparatus

The microstructures of CA and Au-CA were analyzed by scanning electron microscopy (SEM; JEOL JSM-6360, Japan) coupled with energy dispersive spectroscopy (EDS). UV-Vis spectra were recorded on a Nicolet Evolution 300 spectrophotometer in 0.1 M PBS and the IR spectra (KBr) were recorded on an IR-spectrometer FTIR-8400S Shimadzu at room temperature.

### 2.3. The preparation of CA and Au-CA

#### 2.3.1. CA

CA was synthesized by the pyrolysis of RFAs which were produced using the sol–gel route described by Ref. [20]. Molar ratio of resorcinol with respect to formaldehyde was fixed at 1:2 ( $R/F = 1/2$ ), and R/C (resorcinol/catalyst) ratio was 1000. Solvent exchange was performed with acetone 2 times per day for 3 days. Ambient drying was done at room temperature, and CA was prepared by pyrolysis of RF aerogel at  $800^\circ\text{C}$  for 3 h under nitrogen flow.

#### 2.3.2. Au-CA

The preparation procedure for formation of Au-CA was shown in Scheme 1. 95 mg of the prepared CA powder was dispersed into 95 mL EG in a 250 mL beaker by sonicating for 10 min, and then 0.25 mL of  $20\text{ mg mL}^{-1}$   $\text{HAuCl}_4$  solution was dropwise added to the mixing solution above under stirring. After continuously stirred for 30 min, pH value of the mixed solution was adjusted to alkali using 0.1 M NaOH solution. After the resulting solution reacted at  $130^\circ\text{C}$

for 3 h, the solid by filtration was washed with twice-distilled water three times. The resulting solid was dried at  $80^\circ\text{C}$  for 8 h.

### 2.4. Preparation of the modified electrodes

CPE was fabricated as follows: 0.6 g of liquid paraffin and 3.4 g of graphite powder were hand-mixed to produce a homogenous paste. Then the prepared carbon paste was firmly packed into a PVC tube (3 mm internal diameter) and a copper wire (1.5 mm external diameter) was introduced into the other end for electrical contact. The surface of CPE was carefully smoothed on a weighing paper before used.

The preparation procedure of Hb modified electrode was shown in Scheme 1. Firstly, 5 mg of Au-CA and  $10\ \mu\text{L}$  Bmim Br were dispersed into 1 mL 0.1 M pH 7.0 PBS under sonication. Then  $5\ \mu\text{L}$  suspension above was cast onto the surface of a freshly polished CPE to obtain Au-CA/IL-CPE, which was dried at room temperature to form a stable film. Finally, Au-CA/IL/Hb-CPE was prepared by casting  $5\ \mu\text{L}$  Hb solution ( $5\text{ mg mL}^{-1}$ ) onto Au-CA/IL-CPE. The Hb modified electrode was dried at  $4^\circ\text{C}$  in a refrigerator and then rinsed with doubly distilled water for twice or thrice to remove the unimmobilized Hb molecules. When not in use, the Hb modified electrode was stored in 0.1 M pH 7.0 PBS at  $4^\circ\text{C}$  in a refrigerator.

### 2.5. Electrochemical measurements

All electrochemical measurements were carried out with a CHI660B electrochemical workstation (Shanghai Chenhua Co.) controlled by a microcomputer with CHI660 software. A three electrode system was used, where an Ag/AgCl electrode served as the reference electrode (3.0 M KCl, Shanghai Ruosull Tech. Co.), a platinum wire electrode as the auxiliary electrode and a modified CPE as the working electrode. Cyclic voltammetric measurements were done in an undivided 30 mL electrochemical teflon cell at  $25 \pm 0.5^\circ\text{C}$ . Amperometric experiments were performed in a constant stirred cell with the successive addition of  $\text{H}_2\text{O}_2$  or  $\text{NaNO}_2$  into 20.0 mL supporting electrolyte, while the electrode potential was set at  $-350\text{ mV}$  for  $\text{H}_2\text{O}_2$  and  $800\text{ mV}$  for  $\text{NaNO}_2$  vs. RE (Ag). The transient background current was allowed to decay to a steady-state value before adding analytes. Stock solutions of  $\text{H}_2\text{O}_2$  were freshly prepared from 30%  $\text{H}_2\text{O}_2$  solution. All experimental solutions were deoxygenated by purging them with highly pure nitrogen for 30 min and maintained under nitrogen atmosphere during measurements. AC impedance experiments were carried out in 5.0 mM  $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$  (1:1) containing 0.1 M KCl, while the applied perturbation amplitude was 0.005 V, the frequencies swept from  $10^5$  to  $10^{-2}$  Hz, the number of points per frequency decade was 12 and the initial potential was 0.20 V vs. RE (Ag).

## 3. Results and discussion

### 3.1. Characterization of the synthesized Au-CA

EG, as a good reducing agent, has been widely used in the polyol process to prepare metal or metal-based catalysts [21]. Au-CA nanocomposite was prepared by reduction reaction between  $\text{AuCl}_4^-$  and EG in the presence of CA. In this process, EG acted as not only the role of reducing agent but also dispersed CA. The CA and EG were intensively mixed in order that the  $\text{AuCl}_4^-$  ion could be absorbed on the surface of CA. Furthermore, the  $\text{AuCl}_4^-$  was reduced to Au at elevated temperature by mandatory hydrolysis. Finally, the Au was deposited on the surface of CA and partly entered into the pore structure of CA. Thus, Au-CA nanocomposite was obtained.

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