Enzyme-free amperometric sensing of hydrogen peroxide and glucose at a hierarchical Cu$_2$O modified electrode

Song Li, Yajie Zheng, Gaowu W. Qin*, Yuping Ren, Wenli Pei, Liang Zuo

Key Laboratory for Anisotropy and Texture of Materials (MoE), Northeastern University, Shenyang 110819, China

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1. Introduction

Detecting hydrogen peroxide and glucose levels in foods and blood by electrochemical method with a modified electrode has been receiving much attention for many years from chemical, clinical and environmental communities [1–4]. Since the pioneering work by Clark on the enzymatic electrode for determination of glucose level in blood, various enzymatic sensing techniques have been proposed and successfully developed. Despite positive attributes of enzymatic biosensors, the main drawbacks associated with their applications are the insufficient stability originated from the nature of the enzymes and the complexity of enzyme immobilization process, regardless of the employment of artificial mediator or direct electron transfer [5]. Therefore, a growing interest in the development of new-generation electrode without using enzyme for detecting hydrogen peroxide or glucose has been aroused in this field.

To meet the demands of enzymeless sensing, modifying kinds of electrodes by numerous nanostructured materials has been intensively investigated. For instance, to detect glucose, nanoporous and nanotubular Pt [6,7], Ni [8], carbon nanotubes [9], metal oxides [10], and their hybrids [11–13] have been applied to construct modified electrode. Cuprous oxide (Cu$_2$O), as an important p-type semiconductor, demonstrates potential applications in the fields of sensing and solar energy conversion due to its proper redox potentials and excellent stability in air and solutions. The synthesis of Cu$_2$O nanostructures has been reported, including hollow structures [14–16], nanotubes/nanowires [17], and nanocubes [18]. For electrochemical sensing applications, substrate adsorption on electrode surface and prompt charge transfer play essential roles. Control over the morphology of electrode nanomaterial is therefore extremely important [10]. In this work, hierarchical nanocrystals of Cu$_2$O were used to construct an enzyme-free electrode for detecting hydrogen peroxide and glucose. The results showed that a high electrochemical performance was provided by the unique structure.

2. Experimental

The Cu$_2$O nanoparticles were synthesized by a low temperature chemical method, which was similar to literature [16,19]. In a typical procedure, 0.225 g PVP (polyvinyl pyrrolidone, K30) and 0.400 g Cu (CH$_3$COO)$_2$·H$_2$O were dissolved in sequence in 25 ml DMF (N,N-dimethylformamide) at room temperature under stirring. Then 0.100 g NaBH$_4$ was added to the solution and stirring was remained for several minutes until the additives dissolved in the DMF solvent. Then the resultant transparent solution was transferred to a 75 °C water bath and was maintained for 20 min. The modified electrode was prepared as follows. 10 mg of the final Cu$_2$O particles, after washing, was dispersed in 2 ml Nafion solution (0.1%, Sigma–Aldrich). 2.5 µl of the suspension with dispersed Cu$_2$O was cast on pre-treated 3 mm glassy carbon electrode (denoted as Cu$_2$O/Nafion/GCE). Before modification, the bare GCE was polished to a mirror-like surface with 0.3 and 0.05 µm alumina slurry, and then washed ultrasonically in deionized water, 50% (V/V) HNO$_3$ solution, ethanol, and water for a few minutes.
Electrochemical measurements were carried out on a Zahner IM6 electrochemical analyzer with a conventional three-electrode cell. The as-fabricated Cu$_2$O/Nafion/GCE was used as the working electrode with a platinum foil as an auxiliary electrode and a KCl saturated Ag/AgCl reference electrode. The measurements were performed in aqueous phosphate buffer solution (PBS: 0.1 M, pH 7.4). X-ray diffraction (XRD) pattern of the obtained Cu$_2$O nanospheres was collected on an X'Pert PRO diffractometer (Panalytical) with Cu Kα irradiation. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained by employing a JEOL JEM-7100F microscope and a JEOL 2100F microscope.

3. Results and discussion

The morphology of the Cu$_2$O nanoparticles prepared at 75 °C is displayed in Fig. 1a, which shows that the product is composed of uniform nanospheres with an average size of 200 nm and a very narrow size distribution. TEM image (Fig. 1b) reveals that the Cu$_2$O nanospheres are the results of self-assembly of smaller Cu$_2$O nanocrystals. It is worthy of note that, by assembling nanocrystals into a hierarchical structure, the agglomeration of the nanospheres could be prevented [20]. BET specific surface area via nitrogen sorption analysis (Fig. 2a) of the as-synthesized Cu$_2$O is 13.1 m$^2$ g$^{-1}$. Such features of high surface area-to-volume ratio as well as the compact assembling of nanocrystals into polycrystalline spheres provide a very large number of interaction sites for electrochemical reactions and good conductivity [21,22]. Furthermore, as we reported on enhancing the catalytic activity of Pt nanomaterials by linearly assembly of Pt nanoparticles into nanowires [23], grain boundaries are expected to be very helpful for improving the electrochemical performance of the Cu$_2$O nanospheres. The selected area electron diffraction (SAED) ring pattern that corresponds to a small number of nanospheres can be indexed to cubic Cu$_2$O. Further crystallinity and phase information for the product were provided by the X-ray diffraction (XRD) pattern. As shown in Fig. 2, all the diffraction peaks in the pattern match well with the cubic crystal structure of the Cu$_2$O phase (space group: Pn3m, ICDD No. 77-0199). The narrow and sharp peaks in the pattern indicate that the Cu$_2$O nanospheres are highly crystallized, which could have positive effects for the electron transfer in electrochemical reactions.

The electrochemical properties of the electrodes are often characterized by cyclic voltammetry (CV). Fig. 3 shows CV response of Cu$_2$O/Nafion/GC electrode in pH 7.4 PBS at sweeping rate between 25 mV s$^{-1}$ and 1000 mV s$^{-1}$. The cathodic and anodic peak currents can be ascribed to electrochemical reactions of Cu$_2$O, including CuO/Cu$_2$O redox couple and reactions of the produced CuO layer [24].

Fig. 4 The increase of cathodic and anodic peak currents ($i_{pc}$ and $i_{pa}$), as well as small shifts of the cathodic and anodic peak

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**Fig. 2.** (a) Nitrogen adsorption–desorption isothermal curve of the as-synthesized Cu$_2$O nanoparticles. (b) XRD pattern of Cu$_2$O nanoparticles synthesized at 75 °C for 20 min. All diffraction peaks can be indexed to Cu$_2$O (ICDD No. 77-0199).
Fig. 3. Cyclic voltammograms of a Cu$_2$O/Nafion/GC electrode in 0.10 M PBS (pH 7.4) by sweeping at different scan rates. The scan rates from inner to outer are: 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 V s$^{-1}$, respectively.

Fig. 4 shows the dependence of peak current $i$ on scan rate $v$ which can be described by the equation $i = Av^k$, where $A$ is a material-related constant. The parameter $k$ is expected to take a value 0.5 and 1.0 for diffusion-controlled and adsorption-controlled reactions, respectively [3,12,25]. At lower scan rates ($v < 0.60$ V s$^{-1}$), a linear response of peak current on square root of the scan rate was observed, indicating that the electron transfer of Cu$_2$O/Nafion complex with the GC electrode was a diffusion-controlled process. At higher scan rates ($v > 0.6$ V s$^{-1}$), the current peaks increased linearly with the scan rate, suggesting that the electron transfer reaction was controlled by surface adsorption [26]. The switch of a diffusion-controlled behavior to a diffusionless one is because there is not sufficient time for the reaction species to diffuse across the Cu$_2$O/Nafion layers at higher scan rates. Therefore, the reaction products may accumulate on the surface of the electrode. Similar phenomena have been observed on electrocatalytic detection of dopamine on silanized graphene modified GC electrode [27].

Fig. 5a shows the amperometric response of the Cu$_2$O/Nf modified electrode in pH 7.4 PBS upon addition of H$_2$O$_2$ in a 1 μM step at −0.2 V. The response time for detecting H$_2$O$_2$ to obtain a steady current was less than 0.5 s. The calibration curve in the inset shows a highly linear relationship (ca. $R^2 = 0.9991$) between the current and concentration of H$_2$O$_2$ in a broad range below 22 μM, and features a detection sensitivity of 3.693 μA μM$^{-1}$. Calculated...
solution under continuous stirring. Fig. 6a shows a typical response of the electrode to the successive addition of glucose at a working potential of 0.5 vs. Ag/AgCl. The response of the electrode to glucose is rapid and a steady state current signal can be reached within 4 s upon addition of the glucose solution. The corresponding calibration curve for glucose concentration is illustrated in Fig. 6b, which shows a linear relationship range from 0.05 mM to 1.1 mM. The fitting equation is $I = 2.242 + 13.503c$ (mM) with a correlation coefficient of 0.9989. The detection limit was estimated as 47.2 μM at a signal/noise ratio of 3. Anti-interference effect of the modified electrode was tested by adding 0.2 mM glucose, followed with additions of 0.01 mM ascorbic acid (AC), 0.04 mM uric acid (UC) and 0.04 mM NaCl in 0.1 M NaOH solution. The responses shown in inset of Fig. 6a demonstrate that the interference effect from small amount of AC, UC and NaCl can be neglected.

### 4. Conclusion

In summary, spherical Cu2O nanoparticles comprising smaller nanocrystals were synthesized in a simple chemical way. By coating Cu2O/NFs onto GCE surface, enzyme-free sensors were constructed for detecting hydrogen peroxide and glucose. The modified electrodes showed fast amperometric response and high sensitivity. This is because of the increased electroactive surface area and compact attachment of Cu2O nanocrystals resulting from the hierarchical structures. The detection limits for H2O2 and glucose are 0.0039 μM and 47.2 μM, respectively. These properties are believed to be very helpful for developing new biosensors and bio-devices without enzyme.

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