Novel Electrochemical Biosensor for Simultaneous Detection of Adenine and Guanine Based on Cu$_2$O Nanoparticles

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Abstract

In this paper Cu$_2$O nanoparticles were prepared and used for the construction of novel electrochemical voltammetric biosensor for the simultaneous detection of adenine and guanine. Cu$_2$O nanoparticles were synthesized via simple wet chemical route, where glucose was used as a reductant. The nanoparticles were characterized by SEM and XRD analysis, which showed the presence of spherical aggregations with diameter of 1000 nm. These nanoparticles were successfully used for fabrication of spray-coated and screen-printed working electrodes on alumina substrate for the electrochemical detection of purine bases. We observed that Cu(I) reacts with adenine to form insoluble complex that accumulates on the electrode surface and causes the decrease of current response. In the case of guanine, we did not observed any significant decrease of current response which is probably caused by adsorption of guanine on the electrode surface.

Keywords: Thick film technology; electrochemistry; cyclic voltammetry; copper(I) oxide; purines

1. Introduction

Cuprous oxide (Cu$_2$O) is a p-type metal oxide semiconductor with a direct band gap of 2.0–2.2 eV. It has attracted increasing interest due to its promising application in magnetic devices, solar energy conversion and catalysts [1].

New types of solid electrodes are necessary for small device technologies which may replace the standard electrochemical analysis, where toxic mercury drop electrodes are commonly used. The
performance of solid electrode is determined by its surface modification to make it sensitive and selective towards a certain analyte [2]. Solid electrodes can be fabricated using thick–film technology (TFT). The advantage of TFT is its flexibility, low production costs, good reproducibility and good electrical and mechanical properties of electrodes.

Adenine and guanine are components of DNA. Most of the current electroanalytical protocols for DNA detection are based on these electroactive purine bases. The abnormal changes in the concentration of these bases in organisms indicate the efficiency and/or mutation of the immune system, and may be the sign of various diseases. Hence, the determination of individual concentrations of these components, or their ratios in DNA is of great interest in bioscience and clinical diagnosis [3].

2. Experimental

2.1. Cu2O nanoparticles preparation

The preparation method of Cu2O nanoparticles is based on the procedure reported in [4]. Nanoparticles were prepared by two-step synthesis. At first, 0.0035 mol Cu(CH3COO)2•H2O was dissolved in 100 mL absolute ethanol under ultrasonic to form the deep green solution. The solution was heated to 60 °C. Then 50 mL glucose aqueous solution (0.1 mol/L) was slowly added into the solution under vigorous stirring. After that, 50 mL NaOH aqueous solution (0.5 mol/L) was also added to the solution at the same speed. Then some light yellow precipitates was occurred. The particles in the suspension were then separated by centrifugation at 4000 rpm for 8 min. The particles were resuspended in absolute ethanol followed by distilled water. The centrifugation was repeated thrice to remove CH3COO-, glucose and NaOH. The light yellow precipitate was then dried under nitrogen atmosphere overnight.

2.2. Electrodes fabrication

Working mikroelectrodes were fabricated using standard thick-film technology process on the alumina substrate. Thick-film pastes used for contact and covering layers were ESL 9562-G and ESL 4917 both from ESL Electroscience, UK. Carbon paste BQ 221 (Dupont) was screen-printed over Ag/Pd/Pt based contact layer to avoid the contact layer to be present in electrochemical reaction.

The working electrode was fabricated by spray-coating and screen printing deposition using Cu2O nanoparticles powder as the filling material. For spray coating deposition Cu2O particles were dispersed in N-Methyl-Pyrrolidone. To ensure good shape of the electrode a precise template was used for the deposition and the substrate was heated to 250 °C. The spray-coating process was repeated until the carbon layer was totally covered with the nanotubes to prevent later impact to electrochemical analysis. In the case of screen-printed deposition method, Cu2O nanoparticles were well homogenised with vehicle. Prepared paste were screen-printed on an alumina substrate and dried.

2.3. Electrochemical measurement

Electrochemical measurements were performed with PalmSens handheld potentiostat/galvanostat (Palm Instruments BV, Netherlands). The device was connected to a personal computer for measurement setup and response evaluation. A three–electrode system was used, Cu2O electrode was employed as the working electrode, an Ag/AgCl/3M KCl electrode served as the reference electrode and Pt electrode was used as the auxiliary electrode. Cyclic voltammetry (CV) were carried out in the presence of 20 ml 0.2 M acetate buffer pH 5.0 and in the presence of various concentration of adenine or guanine. CV parameters: scan rate 100 mV/s, potential range -0.4 to 0.4 V.
3. Results and discussion

In our experiment, glucose was used as reductant. Reduction of Cu(II) tartrate complex by glucose can yield stable sols of cuprous oxide, whose particle size and morphology are strongly dependent on the concentration of reactants. As shown in Fig. 1, sample has a spherical aggregation with a diameter of 1000 nm. But they are conglomerated by smaller particles with the mean size of 150 nm. According to the XRD measurement, we prepared Cu$_2$O/CuO nanoparticles consisted of 70 % Cu$_2$O and 30 % CuO.

Fig. 1. SEM image of Cu$_2$O spherical aggregations (left) and detail of Cu$_2$O nanoparticles (right).

The electrochemical oxidation of adenine, guanine or other purine derivatives at carbon electrode is well known. Formation of complexes of these compounds with metals including copper has been studied. Cu(II) ions can be reduced to Cu(I) which reacts with adenine to form insoluble compounds that accumulate on the electrode surface and cause the decrease of current response (Fig. 2). This approach can be applied for the detection of oligodeoxynucleotide (ODN) after acid hydrolysis during which the purine bases are released from the ODN chain [5].

Fig. 2. Cyclic voltammograms of Cu$_2$O spray-coating working electrode (left) and screen-printed working electrode (right) in the presence of adenine.
In the case of guanine, we did not observe any significant decrease of current response which is probably caused by adsorption of guanine on the electrode surface (Fig. 3).

4. Conclusion

Cu$_2$O nanoparticles for preparation of thick film pastes were prepared and characterized. Prepared NPs were sprayed or screen-printed on previously prepared electrode substrate. These electrodes were successfully used as the working electrodes for electrochemical detection of adenine and guanine.

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References