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An outstandingly sensitive enzyme-free glucose sensor prepared by co-deposition of nano-sized cupric oxide and multi-walled carbon nanotubes on glassy carbon electrode

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ABSTRACT

An amperometric glucose sensor with excellent sensitivity, very low detection limit and low glucose oxidation potential was prepared by casting of suspension of copper oxide (CuO) nanoparticles/multi-walled carbon nanotubes (MWCNTs) on the surface of a glassy carbon electrode (GCE). Dimethyl formamide (DMF)/H_2O (9:1) was used as a new suspending media. Electrochemical activity of the electrode toward the oxidation of glucose was studied using differential pulse voltammetry, cyclic voltammetry and electrochemical impedance spectroscopy. A synergistic effect was substantiated between the MWCNTs and nano-CuO for glucose electroxidation. The effects of nanocomposite layer thickness and MWCNTs/CuO mass ratio on the sensor performance were investigated and optimized. The detection limit, and linear range of the sensor were identified as 0.07 (±0.03) μmol L^{-1} (S/N = 3) and 0.5–2000.0 μmol L^{-1}, respectively, under applied oxidation potential of 0.35 V (vs. Ag/AgCl). The sensor exhibited excellent sensitivity of 3968.42 (±0.84) μA L $mmol^{-1}$ cm^{-2} to glucose, being higher than those of the previously reported similar sensors. The determination of glucose (5.0 μmol L^{-1}, $n = 5$) with the sensor resulted in RSD% of 3.4. Furthermore, the reproducibility of the sensor was equal to 5.7%. The developed sensor was compared with other enzyme-less glucose sensors and its superiority over them was demonstrated reasonably. The optimized sensor was applied to glucose determination in blood samples. The efficiency of the sensor for glucose determination was comparable with that of a commercial enzymatic sensor.

1. Introduction

Glucose is a consequential metabolite for living organism, especially for clinical diagnostics of diabetes, which is a world-wide public health difficulty and one of the main reasons of death and disability in the world [1,2]. Therefore, glucose sensors development is extremely important. Electrochemical glucose sensors, especially amperometric biosensors, hold a leading position among various biosensors [3,4]. The majority of known amperometric biosensors for glucose monitoring involve the use of glucose oxidase or glucose dehydrogenase enzymes [5,6]. Nonenzymatic glucose sensors based on the direct oxidation of glucose have been proposed as alternative for enzyme-based glucose sensors. Various nanostructured materials have been proposed as new opportunities to develop novel nonenzymatic glucose sensors [7]. Metals [8–10] and metal oxides such as NiO [4,11,12], RuO_2 [13], Co_3O_4 [14], Cu_2O [15] and ZnO [16] were used as the nonenzymatic electrocatalysts to modify the electrodes. The catalytic activity of metals [17] and metal oxides [18] can be greatly enhanced by coupling of them with conducting materials such as carbon nanotubes (CNTs). Among different kinds of metal oxides, copper oxide has attracted special interest because of its cheapness, narrow band gap, durability and etc. Copper oxide has been utilized via different manner for electrocatalytic oxidation of glucose. The CuO nanoplatelets, grown on Cu foil [19], CuO/Nafion-modified GC electrode [20], CuO nanowire-modified Cu electrode [21] and CuO/TiO_2 nanotube arrays electrode [22] have been utilized for fabrication of enzyme-less glucose sensors. None of these electrodes can exhibit all analytical characteristic concurrently acceptable. For example, CuO nanowire-modified Cu electrode exhibits interestingly low oxidation potential but its sensitivity is very low. On the other hand, CuO nanoplatelets/Cu electrode provides higher sensitivity, but it needs higher applied potential for glucose oxidation which increases the risk of interference effect. Efforts to improve the

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glucose oxidation characteristic of CuO have been partly succeed by combination of CNT and CuO on the electrode surface. Based on this strategy, a highly sensitive non-enzymatic glucose sensor has been fabricated by two-step electrodeposition of CuO nanoparticles onto multi-walled carbon nanotube arrays [23]. At another try, cupric oxide has been coated on vertically aligned MWCNTs arrays, on Ta foils, by sputtering method. This work has led to better sensitivity, lower detection limit and lower applied potential, compared to the previous work [24]. These examples indicate that coupling of CuO with MWCNT improves the analytical characteristics of the related sensor. Furthermore, the fabrication of such electrode, requiring the well-aligned MWCNTs on a substrate, is tedious and costly task.

In this work, we presented a new and very simple approach for the preparation of nanoCuO/MWCNTs nanocomposite as a non-enzymatic glucose sensor. For this aim, a mixture of DMF/H_2O (90:1), capable of uniformly dispersion of nanocomposite components, was utilized for deposition of nanoCuO/MWCNTs layer on the surface of glassy carbon electrode. In spite of its simplicity in both design and fabrication, our developed sensor is capable of improvement concurrently all main analytical characteristics of the sensor like sensitivity, detection limit, linear range and applied oxidation potential.

2. Experimental

2.1. Materials

MWCNTs (5–10 nm in diameter, Sigma–Aldrich), having the purity of more than 95%, were used without any pre-treatment. D-(+)-Glucose, dopamine (DA), L(+)-ascorbic acid (AA), uric acid (UA), D-lactose monohydrate (Lac), fructose (Fru), sucrose (Suc), maltose monohydrate (Mal), monopotassium phosphate (KH_2PO_4), sodium chloride (NaCl), sodium hydroxide (NaOH), potassium chloride (KCl) were obtained from Merck (Germany) and used as received. All other reagents were of analytical grade and were purchased from Merck and Sigma–Aldrich (Germany). Double distilled water was used to prepare aqueous solutions. Human blood serum samples were obtained from the Iranian blood transfusion service (Ardabil, Iran).

2.2. Apparatus

Electrochemical experiments were performed with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. The nanocomposite based modified GCE (Metrohm, 0.02 cm^2), platinum wire and Ag/AgCl electrode were utilized as working, counter and reference electrodes, respectively. AUTOLAB PGSTAT302 electrochemical analysis system and GPES 4.9 software package were used for the electrochemical impedance spectroscopy. Impedance measurements were performed at frequency range = 1–5 mHz, ΔE_{ac} = 50 mV and dc potential of 0.35 V (in the presence of glucose (1×10^{-2} mol L^{-1}) and sodium hydroxide (0.1 mol L^{-1})). The measurements were performed in a three-electrode system: counter electrode (platinum), reference electrode (Ag/AgCl) and working electrode (investigated conventional electrodes).

Blood glucose meter device for self testing (model: OK-1B, OK Biotech Co., Ltd.) was utilized as the reference method for the determination of glucose in blood samples in order to evaluate the developed electrode.

2.3. Synthesis of two kinds of CuO nanoparticles

In this work, two kinds of CuO particles were synthesized by sol–gel and alcohothermal methods [25,26]. Briefly, in sol–gel method, CuO material was prepared by thermal decomposition of freshly prepared $Cu(OH)_2$ at temperature of 50 °C. In alcohothermal method, 50 mL of alcoholic solution of copper acetate (0.05 mol L^{-1}) were put into a stainless steel autoclave. The autoclave was then put into an oven and kept constantly under autogeneous pressure at 120 °C for 20 h.

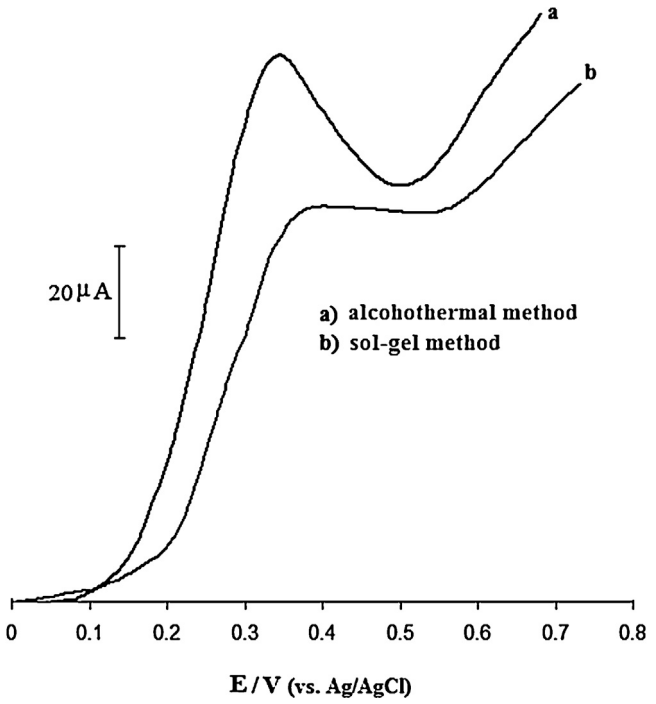

Fig. 1. Differential pulse voltammetry responses of GC electrode, modified with MWCNTs and different CuO particles; differential pulse voltammetry condition: [glucose] = (1×10^{-2} mol L^{-1}), [NaOH] = (0.1 mol L^{-1}), potential range = 0.0–0.8 V, step potential = 0.0055 V, modulation amplitude = 0.050 V.

2.4. Preparation of modified electrode

Before modification, the bare glassy carbon electrode (2 mm in diameter, 0.0314 cm^2) was polished sequentially with alumina powder to create mirror finish. The electrode was then sonicated with absolute ethanol and double distilled water, respectively. It was rinsed thoroughly with double distilled water and then dried under ambient temperature. To fabricate CuO/MWCNTs modified GCE, 6 mg of the CuO nanoparticles and MWCNTs (with optimized mass ratio, mass ratio = m(CuO/mg)/m(MWCNTs/mg)) were dispersed in optimized volume of dimethyl formamide (DMF/H_2O, 9:1) under ultrasonication to give a uniformly dispersed CuO/MWCNTs suspension. Then, 3 μL of this suspension was dropped onto the GCE and allowed to dry in air to form CuO/MWCNTs-modified GCE.

2.5. Glucose determination in real sample

In order to assay the glucose level in human blood, μL amounts of blood samples were injected into 20 mL of NaOH solutions (0.10 M) which were under stirring at 500 rpm. The amperometric signal of glucose was recorded by applying a potential of 0.35 V to the working electrode (developed sensor). The glucose amount in the initial blood samples was then calculated regarding the previously plotted calibration curve and with respect to the dilution order.