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A Nafion-free non-enzymatic amperometric glucose sensor based on copper oxide nanoparticles–graphene nanocomposite

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ABSTRACT

A new and simple method was introduced for the preparation of a non-enzymatic amperometric glucose sensor based on CuO nanoparticles–graphene nanocomposite. Differential pulse voltammetry, cyclic voltammetry and electrochemical impedance spectroscopy were used for the evaluation of the prepared sensors. The synthesized graphene and CuO nanoparticles were dispersed in DMF/H_2O (90:10) solvent mixture and then a little amount of the suspension was drop-coated on the surface of a glassy carbon electrode. It was found that the kind of solvent, utilized for the dispersion of graphene and CuO nanoparticles, had very crucial effect on both sensitivity and reproducibility of the prepared sensor. The nanocomposite coating thickness and CuO/graphene mass ratio were optimized to achieve the best sensitivity. A synergistic effect was substantiated between graphene and CuO nanoparticles for electro-catalytic oxidation of glucose. Dependence of the sensor response to glucose concentration was dynamically linear between 0.5 and 2000 $\mu mol\,L^{-1}$ and at this concentration range the optimized sensor represented very high sensitivity (2939.24 $\mu A\,mM^{-1}\,cm^{-2}$). Moreover, the detection limit of 0.09 $\mu mol\,L^{-1}$ was calculated for this sensor. The sensor was properly utilized for glucose assay in blood samples.

1. Introduction

Glucose measurement has attracted considerable interest due to its potential application in several areas such as the development of blood glucose sensors, wastewater treatment, food industries and environmental monitoring [1,2]. For diabetic patients, regular measurements of blood glucose level are required to confirm whether the treatments are working effectively and to avoid diabetic emergency (blood glucose concentration higher or lower than the normal range of 4.4–6.6 mM) [3]. Thus, there is an ever-growing demand to create highly sensitive, reliably, rapid and low cost glucose sensors [4]. Among different approaches, utilized for the measurement of glucose concentration, such as the optical techniques including infrared spectroscopy, Raman spectroscopy, and photo acoustic spectroscopy [5–8], surface plasmon resonance biosensor [9], capacitive detection [10], electrochemiluminescene [11] and colorimetry [12]. The electrochemical approach has attracted significant attention because of simplicity and high performance. The electrochemical glucose sensors usually have two major types, the enzymatic and non-enzymatic sensors [1]. Enzymatic sensors are highly selective, sensitive, fast and reversible but unavoidable drawbacks such as the chemical and thermal instabilities originated from the intrinsic nature of enzymes as well as the boring fabrication procedures and high cost may limit their analytical applications [13]. To overcome the described problems, great efforts have been made for direct determination of glucose with non-enzymatic sensors. For this purpose, different metal electrodes, such as Pt [14], Au [15], Cu [16] and alloys like Pt–Pb [17] and Pt–Te [18] have been extensively investigated for the development of effective enzyme-less glucose sensors. However, high cost, poor selectivity and instability of these sensors make them unsuitable for practical applications. To avoid these disadvantages and with the great development of nanotechnology, various electrode nanomaterials such as ordered Pt nanotube array [19], Pt nanoparticles [20], Pt–Ru nanoparticles [21], NiPd nanoparticles [22], nanoporous PtPb network [23], PtPb nanowire arrays [24] and three-dimensionally Au film [25] have been applied to modify the electrodes. A short survey in the literature indicates that considerable research has been concentrated on the use of low-cost nanoscale metal-oxide material, such as NiO [26], Co_3O_4 [27], RuO_2 [28], MnO_2 [29] for the preparation of non-enzymatic glucose sensors. Because of the extremely reduced sized, large surface-to-volume ratio, greater level of crystallinity and the Debye length

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comparable to its dimensions, nanostructured metal oxides could greatly enhance sensitivity and selectivity of the related sensors [30].

On the other hand, graphene, a single-atom-thick planer sheet of carbon atom in a closely packed honeycomb two-dimensional lattice, has attracted considerable attention from both the experimental and theoretical scientific communities in recent years [31,32]. In the field of electrochemistry, graphene is considered as a perfect material for the sensing application, since it can play an important part in increasing the performance of sensors due to its high surface area (over 2600 m^2/g) and superior electrical conductivity [33]. Up to now, various metal-oxide-graphene nanocomposites such as Fe_3O_4–graphene [34], MnO_2–graphene [35], Co_3O_4–graphene [36], SnO_2–graphene [37] have been reported as the base for sensor fabrication. Among the metal oxide nanoparticles, CuO has been widely studied because of its applications in catalyst, gas sensors, electrochemistry sensors, lithium ion batteries, etc. [38]. Such sensors are typically fabricated by pasting a mixture of electrochemically active material and conducting polymer binders such as Nafion. However, polymeric binder is not active materials for glucose sensing, which may hinder the electrochemical catalytic sites of the active material, and thus degrade the glucose sensitivity [39].

In this work, we fabricated an effective non-enzymatic glucose sensor based on CuO nanoparticles–graphene nanocomposite modified glassy carbon electrode. Our interest in this paper was to develop a new CuO–graphene modified electrode without using the additive polymer binder in order to improve the sensitivity of the aimed sensor. It was found that the type of solvent, used for the casting of graphene–CuO nanocomposite on the electrode surface was so important in the sensor performance. The described innovations lead to a glucose sensor with superior sensitivity and detection limit over the previously reported similar glucose sensors. The developed glucose sensor was capable to satisfactory assay the glucose level in human blood samples.

2. Experimental

2.1. Instruments and reagents

Surface morphology and distribution of particles were studied via LEO 1430VP scanning electron microscope (SEM), using an accelerating voltage of 15 kV.

Electrochemical measurements were carried out with a three-electrode system using a potentiostat/galvanostat model PGSTAT302 (Metrohm). Glassy carbon electrode (GCE), modified with CuO/graphene nanocomposite was used as the working electrode. A platinum wire and Ag/AgCl electrode were used as the 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 = 5 mHz–1 MHz, ΔE_{ac} = 50 mV and dc potential of 0.3 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.

D-(+)-Glucose, D-lactose monohydrate, sucrose, fructose, maltose monohydrate, L(+)-ascorbic acid (AA), dopamine (DA), uric acid (UA), sodium chloride (NaCl), monopotassium phosphate (KH_2PO_4) were purchased from Merck (Germany). Different solvents, utilized for nanocomposite casting on the electrode surface, including chloroform ($CHCl_3$), tetrahydrofourane (THF), iso-propranolol (i-PRN), N,N dimethyl phosphoric (NMP), dimethyl formamide (DMF), toluene (TLN) and methanol (MeOH) were from Merck (Germany). All other chemicals were of analytical grade and purchased from Merck and Sigma-Aldrich (Germany) and used as received. The used aqueous solvents were prepared using double distillated water. Human blood serum samples were obtained from the Iranian blood transfusion service (Ardabil, Iran).

2.2. Synthesis of graphene

Graphite oxide was prepared by chemical oxidation and exfoliation of natural graphite according to a modified Hummers method [40–42]. Briefly, graphite (2 g) was put into a mixture of concentrated H_2SO_4 (80 mL), $K_2S_2O_8$ (7 g) and P_2O_5 (5 g). The solution was then heated to 80 °C for 5 h [42]. After dilution of the mixture with deionized water, the product was obtained by filtering. The graphene oxide obtained in this stage was then re-oxidized by another oxidation step. For this aim the prepared graphene oxide and $NaNO_3$ (1.0 g) were mixed with H_2SO_4 (95%, 48 mL) in a 250 mL flask. The mixture was stirred for 30 min within an ice bath. Potassium permanganate (6.0 g) was then added gradually to the suspension under vigorous stirring. The ice bath was then removed and the mixture was stirred at 35 °C for 5 h. Then, deionized water (60 mL) was slowly added to the paste with vigorous stirring. The reaction temperature was rapidly increased to 98 °C. The diluted suspension was stirred at 98 °C for 2 h. Finally, H_2O_2 (30%, 20 mL) was added to the mixture. For the purification, the mixture was washed with HCl (5%, w/v) and deionized water several times to obtain the graphene oxide. The obtained sample was collected and dried in vacuum at 60 °C for 6 h. Next, 50 mg of the obtained graphene oxide was dispersed in 20 mL of water by sonication. The graphite oxide was then reduced by adding 0.5 mL hydrazine into the solution, stirred for 4 days at 50 °C. Finally, black hydrophobic powder of graphene was obtained by filtration and dried in vacuum.

2.3. Synthesis of CuO nanoparticles

In this work, CuO nanoparticles were synthesized by a so called alcohothermal methods [43]. Briefly, 50 mL alcoholic solution of copper acetate (0.05 mol L^{-1}) was put into a Teflon-stainless steel autoclave of 60-mL capacity. The autoclave was then put into an oven and kept constantly under autogeneous pressure at 180 °C for 20 h. After the reaction, the autoclave was naturally cooled to the room temperature. The product was recovered by centrifugation; then, the product was washed two times with distilled water and three times with ethanol. A black powder product was obtained after vacuum drying at room temperature.

2.4. Preparation of CuO/graphene modified electrode

For the preparation of modified electrode, glassy carbon electrode was first polished with alumina powder and then rinsed with water and sonicated in ethanol and distilled water in turn. Next, the cleaned GCE was gently blown under a nitrogen stream. Afterwards, 3 μL of suspension of nanoCuO/graphene in DMF/water (9:1) was added onto the cleaned GCE and dried at room temperature.

2.5. Amperometric determination of glucose in real samples

In order to assay glucose amount in blood samples the prepared electrochemical sensor, counter electrode and reference electrode were inserted into NaOH solution (20 mL, 0.1 mol L^{-1}). Afterwards,