



# Amperometric glucose biosensor based on immobilization of glucose oxidase on a magnetic glassy carbon electrode modified with a novel magnetic nanocomposite

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## ARTICLE INFO

### Article history:

Received 27 December 2016

Received in revised form 23 March 2017

Accepted 16 April 2017

Available online 19 April 2017

### Keywords:

Diabetes

Direct electron transfer

Silver nanoparticles

Glucose oxidase

Nanocomposite

## ABSTRACT

We present a method to produce a magnetic glassy carbon electrode (MGCE) modified with multilayer nanocomposite (Ag@MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub>) as an immobilization support to promote electron transfer reactions of glucose oxidase (GOx). The nanocomposite verified by transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and a vibrating sample magnetometer (VSM). The direct electron transfer (DET) between GOx and Ag@MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> nanocomposite was investigated using cyclic voltammetry which showed a pair of well-defined redox peaks corresponding to redox active center of GOx. Utilization of constructed electrode for detection of glucose was studied in N<sub>2</sub> and air saturated solutions (pH 7.0). The GOx/Ag@MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub>/MGCE showed excellent stability, a detection limit of 2.12 μM, a linear range between 6 μM and 2 mM, and a dynamic range up to 2.7 mM at the air saturated solutions. Also, in N<sub>2</sub> saturated solutions (pH 7.0), determination of glucose was carried out by amperometry, and the proposed biosensor showed good reproducibility and stability with a detection limit of 3.8 μM and a linear range between 10 μM and 1 mM. Such analytical performances confirm that the fabricated biosensor used in this work has potential to be applied for development of redox enzyme based biosensors.

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## 1. Introduction

Diabetes mellitus has attracted high attention as public health problem at the worldwide. According to the reports of International Diabetes Federation (IDF) Diabetes Atlas, 342000 people in the Middle East and North Africa Region aged 20–79 died from diabetes in 2015, equivalent to one death every six seconds [1]. The primary emphasis of the diagnosis and management of diabetes focuses on trying to test blood glucose levels one to several times in a day. Consequently, it is necessary to develop the glucose sensors and biosensors for the exact detection of glucose levels at biological samples. In common conventional glucose sensors, enzymatic reactions, mainly based on glucose oxidase (GOx), often play the main

role. In actual, GOx acts as an “ideal” enzyme at glucose biosensors because of its low cost, high specific activity and reliability [2]. Nevertheless, GOx has some drawbacks. GOx belongs to the class of intrinsic redox enzymes with the catalytic center buried deeply inside the protein shell [3]. Thus, it is extremely difficult to obtain electron transfer (DET) between enzymes and electrode surfaces, so that promoters and mediators are needed to obtain their electrochemical responses [4,5]. Consideration to DET of immobilized GOx in biosensor structure is one of biggest challenges at development of new glucose biosensors, which facilitates enabling fast and significance diagnosis at the third-generation glucose biosensors [6]. Third-generation glucose biosensors involve “wiring” an enzyme to the electrode by co-immobilizing the enzyme and mediator directly onto the electrode surface or into an adjacent matrix such as a conductive polymer film [7,8]. The immobilized mediators act as non-diffusion redox relay stations, effectively facilitating the transport of electrons from the enzyme active site to the electrode [9]. In recent years, the fast development of nanotechnology offers new possibilities to design and fabricate mediator materials

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with high performance to promote DET [10]. Numerous studies also found that nanomaterials of various shapes, sizes, and compositions could improve the performance of electrochemical biosensors and significantly increase the electrical conductivity [11–13]. With manipulated composition and surface modification, nanomaterials such as, magnetic particles [14,15], metal nanoparticles [16,17] and carbon nanotubes (CNTs) [18,19] can be used in fabrication of novel biosensors. In consequence of developments within nanotechnology, the application of metallic nanoparticles in sensing products is rapidly increasing [20]. Among the metallic nanoparticles, silver nanoparticles (AgNPs) are being used in numerous fields due to their conductive and antimicrobial properties [21,22]. The releases of Ag<sup>+</sup> ions from silver rich surfaces produce significant antibacterial and cytotoxic effects in several biological medium. There are over 438 products available on the open market that contain AgNPs [20,23]. In recent years, AgNPs have been widely employed for the improvement of sensing properties in the sensors and biosensors [24], development in the detection of DNA [25] and cancer [26]. In the last two decade, numerous studies have utilized CNTs in bioapplications ranging from the cancer biomarkers [27] to biosensing applications for a host of biomolecules [28] and even for the field of genetics [29]. Literature survey have proved that the combination of CNTs with nanomaterials such as nanoparticles, thus developing CNTs-nanoparticle hybrid nanostructures, presents several extra unique physicochemical properties and utilities that are both highly necessary and noticeably helpful for bio-applications compared with either material alone [30–32]. Furthermore, such matrices can well become incorporated in a clinical and laboratory diagnostic tools because they have some advantages such as high surface area, unique catalyst activities, good stability and excellent electrical conductivity [33]. Fe<sub>3</sub>O<sub>4</sub> nanoparticles, also known as biomolecule carriers, are typically magnetic nanoparticles with biosensing applications [31]. Extensive research efforts were recently directed towards the application of Fe<sub>3</sub>O<sub>4</sub> nanoparticles as magnetic loaders with the help of external magnetic field. In biosensing platforms, the synergic effect of nanoparticles with other material, which have excellent conductivity and catalytic properties, can provide the necessary conduction pathways for electrons on the electrode surface to increase current signal. Room temperature ionic liquids (ILs) are very good candidate that can be applied in electrochemical biosensors and have presented superior electrochemical performances as a result of their profit, such as good conductivity, wide electrochemical windows, low volatility, appropriate chemical properties and good thermal characteristic [34]. The functionalization of Fe<sub>3</sub>O<sub>4</sub> nanoparticles with ILs opens a new way to development of novel and efficient approaches for the combination of the very small building blocks of biosensors into desired structures. To the best of our knowledge, there are few well-defined reports about such structures.

In the present work, we report a novel multilayer nanocomposite Ag@MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> for fabrication of third-generation biosensors. MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> composite has been consisted from MWCNTs orderly assembled on functionalized positions of Fe<sub>3</sub>O<sub>4</sub> nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs) with ILs. The main novelty of our MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> nanocomposite mainly includes the following points: (1) the high magnetic properties of MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> nanocomposites makes it as a good matrix for stable immobilization on magnetic glassy carbon electrode (MGCE) surface, and (2) functional groups on the MWCNT are ideal reaction sites for the electrodeposition of other nanoparticles (here AgNPs) and thus provide an appropriate approach for enzyme immobilization. Herein, the proposed Ag@MWCNT-IL-Fe<sub>3</sub>O<sub>4</sub> nanocomposite supplied a unique interfacial microenvironment for GOx, which could accelerate the DET at the electrode surface.

## 2. Experimental section

### 2.1. Materials

The chemicals, GOx (Aspergillusniger, EC 1.1.3.4. 150,000 unit/g) and glucose (Sigma, 99%) were obtained from USA and used without additional purification. Ferric chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O), ferrous chloride tetrahydrate (FeCl<sub>2</sub>·4H<sub>2</sub>O), and all solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. The multi-walled carbon nanotubes (MWCNTs, 10–15 nm outer diameter, 2–6 nm inner diameter and 0.1–10 μm length), 3-bromopropan-1-amine and triethanolamine were obtained from the Sigma–Aldrich (St. Louis, MO, USA). All the supplementary chemicals were of analytical grades and solutions were prepared with deionized water. The phosphate buffer solution (PBS) was prepared from phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) and dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) and adjusting the pH was controlled with hydrochloric acid (HCl) and potassium hydroxide (KOH) solutions.

### 2.2. Apparatus and instrumentations

Autolab electrochemistry instruments (Autolab, Eco Chemie, Netherlands) was used for amperometry measurements and electrochemical impedance spectroscopy (EIS). Cyclic voltammetry (CV) measurements were carried out on a Metrohm (797 VA Computrace, Switzerland) controlled by a personal computer. A saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode were used. Fourier transform infrared spectroscopy (FTIR) data were recorded on a Perkin Elmer GX FTIR spectrometer. The structure and the morphology of the samples were characterized using a digital scanning electron microscope (SEM) (Model KYKY-EM3200, KYKY, China) and a Hitachi HT-7100 (100 kV) transmission electron microscope (TEM). The magnetic properties were analyzed with vibrating sample magnetometer (VSM) (LDJ 9600-1, USA). X-ray diffraction (XRD) patterns were obtained on a powder X-ray diffraction system from PANalytical model X'Pert PRO (PANalytical B.V., Almelo, The Netherlands). A digital pH-meter (780 pH meter, Metrohm) with precision of ±0.001 was used to read the pH value of the buffer solutions. All experiments were performed at room temperature (25 ± 2 °C). Electrolyte solutions were deoxygenated by purging pure nitrogen (99.99%) for 10 min prior to electrochemical experiments.

### 2.3. Synthesis process

#### 2.3.1. Preparation of the magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles

Magnetic iron oxide nanoparticles were synthesized according to a previous report [31]. In brief, FeCl<sub>3</sub>·6H<sub>2</sub>O (5.838 g, 0.0216 mol) and FeCl<sub>2</sub>·4H<sub>2</sub>O (2.147 g, 0.0108 mol) were dissolved in 100 mL deionized water at 85 °C under N<sub>2</sub> atmosphere and vigorous mechanical stirring (500 rpm). Then, 10 mL of 25% NH<sub>4</sub>OH was quickly injected into the reaction mixture in one portion. The addition of the base to the Fe<sup>2+</sup>/Fe<sup>3+</sup> salt solution resulted in the formation of the black precipitate of Fe<sub>3</sub>O<sub>4</sub> NPs immediately. The reaction continued for another 25 min and the mixture was cooled to room temperature. Subsequently, the resultant ultrafine magnetic particles were treated by magnetic separation and washed several times by deionized water.

#### 2.3.2. Preparation of amine functionalized IL

The amine functionalized IL, 3-amino-*N,N,N*-tris(2-hydroxyethyl)propan-1-aminium bromide (amine-IL), was prepared by stirring 3-bromopropan-1-amine (1.0 mmol) and triethanolamine (1.0 mmol) in 5 mL methanol at room temper-

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