Available online at www.sciencedirect.com
ScienceDirect
Procedia Chemistry 19 (2016) 50 – 56
Procedia Chemistry

5th International Conference on Recent Advances in Materials, Minerals and Environment (RAMM) & 2nd International Postgraduate Conference on Materials, Mineral and Polymer (MAMIP), 4-6 August 2015

Study of ITO glass electrode modified with iron oxide nanoparticles and Nafion for glucose biosensor application

Noorhashimah Mohamad Nor[a], Zainovia Lockman[a], Khairunisak Abdul Razak[a]§

[a]*School of Materials and Mineral Resources Engineering Universiti Sains Malaysia, Nibong Tebal, Penang 14300 Malaysia*

Abstract

In this study, we report the fabrication of the indium tin oxide (ITO) glass electrode modified with iron oxide nanoparticles (IONPs) and nafion for glucose biosensor applications. The IONPs was synthesized using the precipitation method and functionalized with citric acid (CA) to provide hydrophilic surface and functional group for glucose oxidase (GOx) enzyme immobilization. The structural and morphological studies of CA-IONPs were characterized using X-ray diffractometer (XRD) and transmission electron microscope (TEM). The size of the IONPs measured from TEM image was ~ 17 nm. The bioelectrode designated as Nafion/GOx/CA-IONPs/ITO was developed by drop casting of the CA-IONPs, GOx and nafion on the ITO glass. The Nafion/GOx/CA-IONPs/ITO bioelectrode showed good electrochemical performance for glucose detection. The functionalized CA-IONPs acted as the catalyst and help to improve the electron transfer rate between GOx and ITO electrode. In addition, thin nafion film was coated on the electrode to prevent interference and improve chemical stability. The Nafion/GOx/CA-IONPs/ITO bioelectrode showed high sensitivity of 70.1$\mu AmM^{-1}cm^{-2}$ for the linear range of 1.0-8.0 mM glucose concentrations.

Peer-review under responsibility of School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia

*Keywords:*Iron oxide nanoparticles; glucose biosensor; citric acid; nafion

Nomenclature

ITO indium tin oxide

* Corresponding author. Tel.: +604-5996126 ; fax: +604-5941011.
*E-mail address:*khairunisak@usm.my

Peer-review under responsibility of School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia
doi:10.1016/j.proche.2016.03.116

IONPs	iron oxide nanoparticles
CA	citric acid
GOx	glucose oxidase
FeCl	iron (II) chloride
NaOH	sodium hydroxide
H_2O_2	hydrogen peroxide
PBS	phosphate buffer saline
RCA	Radio Corporation America
XRD	X-ray diffraction pattern
TEM	transmission electron microscopy
CV	cyclic voltametry

1. Introduction

Diabetes is a world-wide public health problem that is a leading cause of death and disability in the world. The diagnosis and management of diabetes mellitus require a tight monitoring of blood glucose[1]. Therefore, a simple and low-cost method that can be used at home to monitor blood glucose level is required. For that, glucose electrochemical biosensors are widely used. Generally, the glucose biosensor is based on the GOx enzyme. The glucose can be detected through measuring the increament of the anodic current during the oxidation of hydrogen peroxide (H_2O_2) produced from the oxidation of glucose by dissolved oxygen in the presence of GOx enzyme[1-2]. However, it is difficult for an enzyme (protein) to exchange electrons directly with bare solid electrodes due to its insulation-shelled redox center[2-4]. Therefore, enzymes were incorporated with metal nanoparticles to allow direct electron transfer.

Among various metal nanoparticles, IONPs have recently gained interest in glucose biosensor applications due to their properties of chemically and biological inert, low toxicity and super paramagnetic. It was observed the existing problems of IONPs are the agglomeration due to high volume to surface area of the IONPs that tend to attract them together in order to minimize their high surface energies[4]. The agglomeration can be prevented by functionalization of the IONPs with organic, inorganic and biopolymeric material such as chitosan, silica, polymers and carbon[5]. Among them, small molecules like citric acid and oxalic acid could be more suitable for IONPs functionalization due to their short chain tricarboxylic acid[6]. The carboxylate group presence may prevent particles agglomeration, provide surface hydrophilic and provide functional group for biomolecule attachment[6-8]. Deb et al.[7] have fabricated citric acid functionalize IONPs by using the co-precipitation method. IONPs produced showed less aggregation and contained anti-platelet activity to be used as the drug carriers in the treatment hyperactive platelets. Recently Sharma et al.[8] reported the efficient immunosensor for diarrhea and acidosis by utilizing IONPs functionalized CA for electrode modification. The CA-IONPs provided more specific surface area for larger biomolecule binding and the magnetic force attraction has improved the biosensing properties. Nafion encapsulation of enzyme is a common practice to prepare biosensors. Nafion is a sulfonatedtetrafluorethylene copolymer that has been widely used as a proton conductor for proton exchange membrane in biosensor applications. The main advantages of Nafion in biosensor applications are its biocompatibility, excellent thermal and mechanical stability, mechanical strength, and antifouling properties.

In this work, the sensing performance of IONPs functionalized with CA for glucose sensing application was evaluated. CA-IONPs were synthesized and drop casted on the ITO glass electrode. To the best of our knowledge, there is no work reported on the CA-IONPs used in electrode modification for glucose sensing. Here, the electrochemical and electrocatalytic performance of the Nafion/GOx/CA-IONPs/ITO bioelectrode in glucose sensing was evaluated. The surface functionalization of CA on the IONPs prepared the favourable microenvironment for biomolecule loading, prevent agglomeration between IONPs and increase electron mobility between the analyte and bioelectrode.

2. Materials and Methods

2.1. Synthesis of CA-IONPs

The precipitation of IONPs was carried out in a reactor. 0.3 M iron (II) chloride ($FeCl_2$) and 1 M sodium hydroxide (NaOH) were pumped in simultaneously into 600 ml of water over 10 minutes with continuous stirring under nitrogen gas atmosphere[9]. The pH of the solution was maintained at pH 8.0 throughout the reaction by using a titrator. The precipitates formed were oxidized using 0.4 M hydrogen peroxide (H_2O_2). The colour of solution changed from milky green to black indicating the formation of IONPs. The precipitates then were allowed to crystallize completely for another 2 hours under mechanical stirring. Then, the precipitates were collected using a magnet and the supernatant was discarded. The precipitates were washed with 1 litre water and peptized overnight using 1.25 M CA. Second peptization was carried out for 3 hours using 1.25 M CA and finally washed with distilled water. The CA-IONPs produced were collected, dispersed in water and pH was adjusted to ~ pH7. The ferrofluids produced were characterized using X-ray diffractometer (XRD) (P8Advan-Bruker with Cu-K_α radiation source) to determine phase's presence. Transmission electron microscope (TEM) (Philips CM12.Version 3.2) was used to determine the size and distribution of IONPs.

2.2. Fabrication Nafion/GOx/CA-IONPs/ITO

ITO glasses were cut and cleaned using alkaline Radio Corporation America (RCA) to improve the wettability. The ITO glass was immersed in ammonium hydroxide (NH_4OH), H_2O_2 and distilled water in a ratio of 1:4:20 at 60 °C for 20 min, and then rinsed with distilled water. In order to eliminate water traces, Isopropyl alcohol was used and dried in a nitrogen gas flow[10]. Then, 100 µL CA-IONPs (1 mg/ml) was dropped on the ITO glass and dried in an oven at 80 ºC for 2 hours. After that, 20 µL GOx (1 mg/ml) was immobilized and kept at 4 °C overnight. Finally, 10µL of 5% nafion was dropped on the biosensor to protect the enzyme layer and to provide good electrical conductivity. All prepared bioelectrodes were stored in dry condition at 4 °C when not in use. The electrochemical performance (cyclic voltammetry (CV) measurements) of the bioelectrode were conducted using an Autolab Potentiostat/Galvanostat with three-electrode cell where samples were used as working electrode, platinum electrode as the auxiliary electrode and Ag/AgCl as the reference electrode in 0.1 M phosphate buffer saline (PBS) (pH 7.0) as the redox probe. All the measurements were performed at room temperature.

3. Result and Discussion

3.1 Characterization of synthesized IONPs

Fig. 1 shows the XRD pattern of the as synthesized IONPs and CA-IONPs corresponded to the peaks of spinel cubic lattice of maghemite ascribed to cubic γ-Fe_2O_3(JCPDS No.: 00-039-1346). This result was expected due to the oxidation process conducted using hydrogen peroxide (H_2O_2) and the peptization process of IONPs using acids. In the presence of acid, maghemite was obtained instead of magnetite due to the oxidation of Fe^{2+} ions to Fe^{3+} ions and left behind the lattice vacancy[11]. Extra vacancies in the γ-Fe_2O_3 structure are thought to be found in octahedral positions. No additional peaks were observed, indicating the formation of a pure and single phase without impurities that remained from the un-reacted precursors.

Fig. 2 shows the TEM image of the CA-IONPs and their size distribution. The CA-IONPs produced through the precipitation of ferrous salt were slightly spherical in shape with mode particle size of 17.3 nm. This was most likely due to the fact that the nucleation rate per unit area was isotropic at the interface between the magnetic nanoparticles[11].Nucleation occurs when the concentration of iron (II) reaches the supersaturation and then proceeds with the growth of the nuclei through the diffusion of solutes from the solution to their surface until the final size is attained.

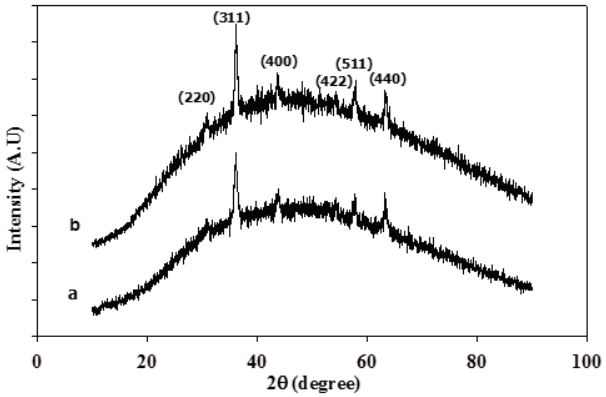

Fig.1. (a) as synthesized IONPs (b) CA-IONPs

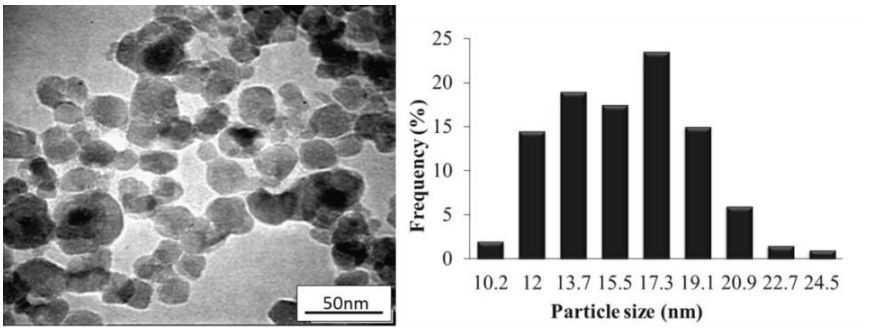

Fig. 2. TEM image of CA-IONPs and their size distribution

3.2 Electrochemical behavior of bioelectrode

CV analysis was used to investigate the electrochemical characteristics of the electrodes because the electron transfer between the electrolyte and electrode must occur by tunnelling through either barrier or defects. Fig. 3 shows the CVs of modified ITO electrodes in the potential range -0.8 to 0.4 V in 0.1 M PBS (pH7.0) with a scan rate of 100 mV/s. At the GOx/ITO (Fig. 3 (a)), there is no oxidation or reduction peak observed due to the embedded enzyme redox center for electron transfer. When the ITO electrode was modified with CA-IONPs, a well defined redox anodic and cathodic peak was observed (Fig. 3 (b)). CA-IONPs has increased electron mobility at the electrode surface resulting in enhanced electron transfer. However, when GOx enzyme and nafion incorporated in the bioelectrode Nafion/GOx/CA-IONPs/ITO (Fig. 3 (c)) peak current decreased. This occurs due to the non conductive enzyme molecules and semi permeable properties of nafion that obstruct the transfer of electrons from electrolyte to electrode surface. This result is in agreement with Sharma et al.[8] and Chen et al. [1].

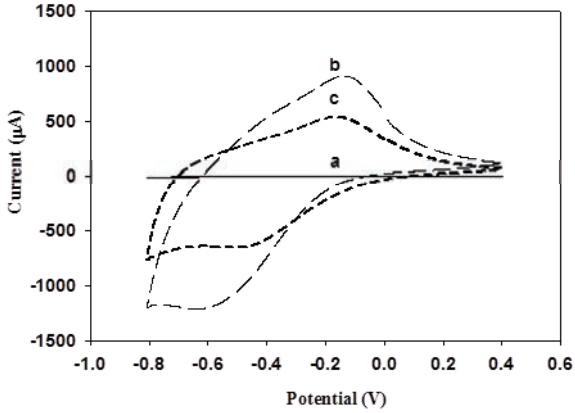

Fig. 3. CVs of (a) GOx/ITO, (b)CA-IONPs/ITO, (c)Nafion/GOx/CA-IONPs/ITO electrodes in 0.1 M PBS (pH7) at scan rate of 100 mV/s

Fig. 4 shows the CVs of CA-IONPs/ITO (Fig.4 (a)) and Nafion/GOx/CA-IONPs/ITO (Fig.4 (b)) electrode in 0.1 M PBS at various scan rates (30-250 mV/s). It was observed in all the electrodes that both the anodic (I_{pa}) and cathodic (I_{pc}) peaks current increases with increasing scan rate, suggesting that the electrochemical reaction is a diffusion-controlled process. The surface concentrations of the CA-IONPs/ITO and Nafion/GOx/CA-IONPs/ITO bioelectrode were calculated using Brown–Anson model by the following equation[13]:

$$I_p = \frac{n^2 F^2 I^* AV}{4RT} \tag{1}$$

where n is the number of electrons transferred which is 2 in this case, F is the Faraday constant (96,485 $Cmol^{-1}$), I* is the surface concentration of the ionic species of the bioelectrode($molcm^{-2}$), A is the surface area of the electrode ($1cm^2$), R is the gas constant (8.314 $Jmol^{-1}$ K^{-1}),T is the absolute temperature (298 K) and Ip/V is the slope of the calibration plot. The surface concentration of CA-IONPs/ITO and Nafion/GOx/CA-IONPs/ITO bioelectrodes was found to be 1.27 x 10^{-10} M cm^{-2} and 2.36 x 10^{-11} M cm^{-2} respectively. The results indicate that after immobilization of GOx enzyme and nafion film, the surface concentrations decrease which confirms the presence of the enzyme and nafion on the ITO biolectrode.

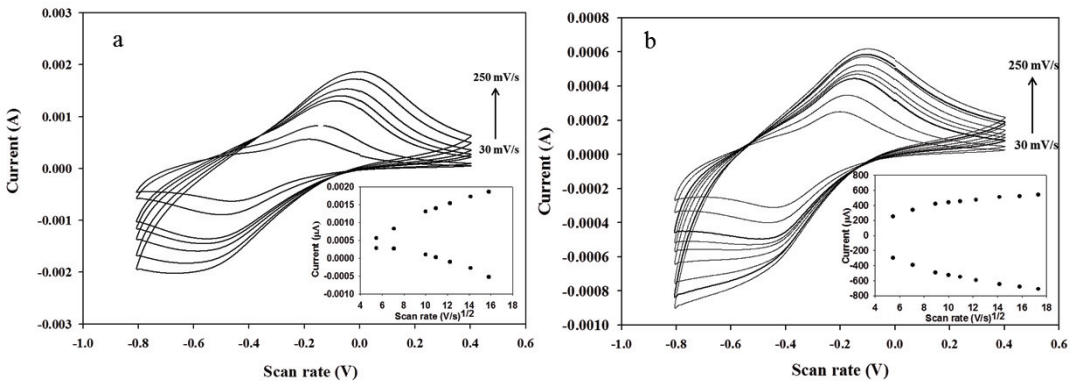

Fig. 4. CVs of (a) CA-IONPs/ITO (b) Nafion/GOx/CA-IONPs/ITO electrodes in 0.1 M PBS (pH7) at scan rate from 30-250 mV/s. The inset shows the calibration curve of current vs scan rate $^{1/2}$

3.3 Electrocatalytic oxidation of glucose at modifiedbioelectrode

Fig. 5 shows results of the CV studies carried out to investigate the activity of the Nafion/GOx/CA-IONPs/ITO bioelectrode as a function of glucose concentration (0-8 mM) in 0.1 M PBS buffer (pH 7) at a scan rate of 100 mV s^{-1}. In the absence of glucose (curve a), typical oxidation and reduction peaks was observed. In 1 mM of glucose (curve b), the oxidation current peak increases displaying a pronounce of electrocatalytic behavior of the GOx enzyme presence on the electrode. The mechanisms of electrochemical behavior of the Nafion/GOx/CA-IONPs/ITO bioelectrode are summarized as follow:

$$\text{Glucose} + \text{GOx} \rightarrow \text{Gluconolactone} + \text{GOx(r)} \quad (2)$$

$$\text{GOx(r)} + O_2 \rightarrow \text{GOx} + H_2O_2 \quad (3)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (4)$$

During the reoxidation of GOx after enzymatic reaction, the CA-IONPs accept electrons from the reduced enzyme, thereby causes increment in the oxidation current. The magnitude of the oxidation peak increases linearly with the increase in the glucose concentration (Fig. 5). It is revealed that Nafion/GOx/CA-IONPs/ITO bioelectrode (inset of Fig. 5) can be used to estimate glucose from 1.0 – 8.0 mM. The sensitivity of the Nafion/GOx/CA-IONPs/ITO bioelectrode calculated from the slope of curve was found to be 70.1µA$mM^{-1}cm^{-2}$ with linear regression 0.97. This result is comparable to other work, which commonly used composite to modify the glucose biosensor electrode[1, 14-16].

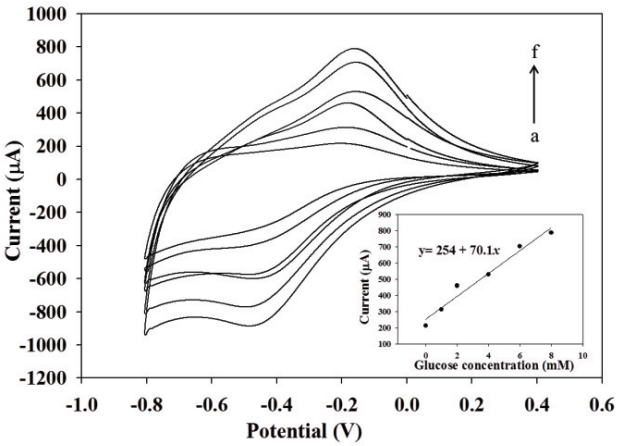

Fig. 5.CVs of Nafion/GOx/CA-IONPs/ITO biolectrode (a) in absence of glucose (b-f) in 1, 2, 4, 6 and 8 mM glucose into 0.1 M PBS (pH7.0) at scan rate 100 mV/s. Inset: Calibration curve of current obtain as function of glucose concentration

4. Conclusion

GOx enzyme, CA-IONPs and nafion were successfully modified the ITO bioelectrode for glucose sensing. CA prevented the aggregation of IONPs without changing its optical and electrical properties. The immobilized GOx displayed excellent catalytic property to glucose and CA-IONPs in the biosensing interface offered not only friendly

environment to immobilize GOx but also improved the electron transfer between analyte (glucose) and CA-IONPs/ITO electrode surface. Nafion/GOx/CA-IONPS/ITO bioelectrode showed high sensitivity to glucose sensing but further testing related to the bioelectrode stability, interference and reproducibility were required. Because of its convenient preparation and good properties, this proposed method could be extended to other enzymes and bioelectrode development for biosensing applications.

Acknowledgements

The authors appreciate the technical supports from the School of Materials and Mineral Resources, and Institute for Research in Molecular Medicine (INFORMM), UniversitiSains Malaysia. This research was funded by TRGS 203/PBahan/6763001. One of the authors acknowledge financial support by MyBrain.

References

1. Chen X, Zhu J, Chen Z, Xu C, Wang Y, Yao C. A novel bienzyme glucose biosensor based on three-layer Au–Fe_3O_4@SiO_2 magnetic nanocomposite. *Sens. Actuator. B* 2011;**159**:220-228
2. Lili Y, Hui W, Beina W, Ziyi W, Hongmei C, Congying F, Nengqin J. Magnetic Fe_3O_4-reduced graphene oxide nanocomposites-based electrochemical biosensing. *Nano-Micro Letters* 2014;**6**:258-267.
3. Li J,Yuan R, Chai Y. Simple construction of an enzymatic glucose biosensor based on a nanocomposite film prepared in one step from iron oxide, gold nanoparticles, and chitosan. *Microchim. Acta* 2011;**173**:369-374.
4. Kaushik A, Khan R, Solanki PR, Pandey P, Alam J, Ahmad S, Malhotra BD. Iron oxide nanoparticles–chitosan composite based glucose biosensor. *Biosens. Bioelectron 2008*;**24**:676-683
5. Sahoo Y, Goodarzi A, Swihart MT, Ohulchanskyy TY, Kaur N, Furlani EP, Prasad PN. Aqueous ferrofluid of magnetite nanoparticles: fluorescence labeling and magnetophoretic ontrol. *J. Phys. Chem. B* 2005;**109**: 3879-3885.
6. Nigam S, Barick KC, Bahadur D. Development of citrate-stabilized Fe_3O_4 nanoparticles: conjugation and release of doxorubicin for therapeutic applications. *J. Magn. Magn. Mater*2010;**323**:237-243.
7. Deb S, Raja SO, Dasgupta AK, Sarkar R, Chattopadhyay A.P, Chaudhuri U, Guha P, Sardar P. Surface tunability of nanoparticles in modulating platelet functions. *Blood Cells, Molecules, and Diseases* 2011;**48**:36-44.
8. Sharma A, Baral D, Bohidar HB, Solanki PR. Oxalic acid capped iron oxide nanorods as a sensing platform. *Chemico-Biological Interactions* 2015;**238**,129-137.
9. Mohamad Nor N, Abdul Razak K, Tan TS, Noordin R. Properties of surface functionalized iron oxide nanoparticles (ferrofluid) conjugated antibody for lateral flow immunoassay application. *J. Alloys Compd*. 2012;**538**:100-106.
10. Besbes S, Ouada HB, Davenas J, Ponsonnet L, Jaffrezic N, Alcouffe P. Effect of surface treatment and functionalization on the ITO properties for OLEDs. *Mater. Sci. Eng. C*. 2006;**26**:505-510
11. Alibeigi S, Vaezi MR. Phase transformation of iron oxide nanoparticles by varying the molar ratio of Fe^{2+}:Fe^{3+}.*Chem. Eng. Technol.* 2008;**31**: 1591-1596.
12. Wang FH, Yoshitake T, Kim DK, Muhammed M, Bjelke B, Kehr J. Determination ofconjugation efficiency of antibodies and proteins to the superparamagnetic iron oxide nanoparticles by capillary electrophoresis with laser-induced fluorescence detection. *J.Nanopart. Res.* 2003;**5**: 137–146.
13. Solanki PR, Arya SK, Singh SP, Pandey MK, Malhotra BD. Application of electrochemically prepared poly-N-methylpyrrole-p-toluene sulphonate films to cholesterol biosensor. *Sens. Actuators B* 2007;**123**:829-839
14. Yang L, Ren X, Tang F, Zhang L. A practical glucose biosensor based on Fe_3O_4 nanoparticles and chitosan/nafion composite film. *Biosens. Bioelectron*. 2009; **25**:889-895.
15. Kang X, Mai Z, Zou X, Cai P, Mo J. A novel glucose biosensor based on immobilization of glucose oxidase in chitosan on a glassy carbon electrode modified with gold-platinum alloy nanoparticles/multiwall carbon nanotubes. *Anal. Biochem.* 2007;**369**: 71–79.
16. Zhao G, Xu JJ, Chen V. Fabrication, characterization of Fe_3O_4 multilayer film and its application in promoting direct electron transfer of haemoglobin.*Electrochem. Commun*2006;**8**:148–154.