THE EFFECT OF IRON OXIDE NANOPARTICLES CONCENTRATION ON THE ELECTROCATALYTIC PERFORMANCE OF ENZYMATIC GLUCOSE BIOSENSOR

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ABSTRACT

In this study, the enzymatic glucose biosensor was developed by modifying indium tin oxide (ITO) glass electrode with iron oxide nanoparticles (IONPs), glucose oxidase (GOx) enzyme and nafion. IONPs were synthesized using a precipitation technique and surface functionalized using citric acid (CA). The effect of iron oxide nanoparticles (IONPs) concentration (1mg/ml to 4 mg/ml) and nafion to the electrochemical performance of the modified electrode for glucose biosensor detection was studied. Increasing the concentration of IONPs increases the electrochemical performance of the bioelectrode. From the transmission electron microscopy (TEM) images, the size of IONPs obtained was ~19 nm and the X-ray diffraction (XRD) spectra showed the presence of spinel cubic lattice of maghemite (γ-Fe₂O₃). The bioelectrode designated as Nafion/Gox/IONPs-CA/ITO shows good electrochemical performance for glucose detection with high sensitivity of 90.44 µA mM⁻¹ cm⁻² for a linear range of 1.0-12.0 mM glucose concentration.

Keywords: Iron oxide nanoparticles; glucose biosensor; nafion; enzymatic biosensor;

INTRODUCTION

Blood glucose monitoring has been established as a valuable tool in the management of diabetes. Therefore fast, sensitive and reliable glucose biosensor has attracted considerable attention in clinical diagnosis recently [1]. For that, the enzymatic electrochemical glucose biosensors have been widely used. Glucose oxidase (GOx) could be immobilized easily on the electrode surface via drop casting method to develop glucose biosensor. However, it is difficult for enzymes to exchange electron directly with bare electrodes surface due to embedded redox center. Another problem is that the shape of the enzyme might change when the enzyme is adsorbed on the electrode surface [2,3]. Therefore, the immobilization of GOx on a suitable matrix is an important factor in fabrication of glucose biosensors.

The modification of the bioelectrode with IONPs has been considered interesting for immobilization of GOx because of their properties that are chemically inert,
biocompatible, strong superparamagnetic property and low toxicity [4,5]. Modification of the electrode with IONPs will promote strong absorption ability of the GOx enzyme and allow direct electron transfer between enzyme and electrode [6]. However, the main problem dealing with IONPs is the agglomeration that usually occurs due to their high surface energy [5]. It can be overcome by surface functionalize the IONPs with organic, inorganic and biopolymeric material such as chitosan, silica, polymers and carbon [7-8]. Among them, small molecules like citric acid could be more suitable for functionalization of IONPs due to their short chain tricarboxylic acid [9]. Citric acid functionalization will provide IONPs surface with carboxylate functional group to prevent agglomeration, provide surface hydrophilic and provide functional group for enzyme attachment [9-10]. Previously Cheraghipour et al. (2012) [11] reported the IONPs functionalized CA for hyperthermia therapy. The carboxylic acid terminal group not only render the particles more water dispersible but also provides a site for further surface modification. Recently, Sharma et al. (2015) [12] reported the efficient immunosensor for diarrhea and acidosis by utilizing IONPs functionalized CA for electrode modification. The CA-IONPs provide more specific surface area for larger biomolecule binding and the magnetic force attraction has improved the biosensing properties. Nafion films have been used extensively for the construction of biosensors to prevent loss of the enzyme molecules and to improve the anti interferent ability of the biosensor. Norouzi et al. (2010) [13] reported the study of glucose biosensor based on carbon nanotubes and gold nanoparticles in a nafion film. The nafion film is used for protective coating material and as a support for enzyme immobilization.

In this work, IONPs were initially synthesized and functionalized with CA. Then the CA-IONPs were drop cast on the ITO glass electrode to develop an enzymatic glucose biosensor. IONPs-CA acted as the matrix for GOx enzyme immobilization and increased the electron mobility between analyte and bioelectrode. The effect of IONPs concentration to the electrochemical and electrocatalytic performance of the Nafion/GOx/IONPs-CA/ITO bioelectrode was evaluated. Increasing the concentration will increase the electrochemical performance of the bioelectrode due to increasing number of CA-IONPs present that allows electron transfer. To the best of our knowledge, there is no work reported on the CA-IONPs used in modification of electrode for glucose biosensor.

**EXPERIMENTAL**

*Synthesis of CA-IONPs.*

IONPs was synthesized via a precipitation technique in a reactor. 0.3 M Iron (II) chloride (FeCl₂) and 1 M sodium hydroxide (NaOH) were pumped in simultaneously into 600 ml degassed water over 10 minutes with continuous stirring under nitrogen gas atmosphere [14]. The pH of the solution was maintained at pH 8 throughout the reaction by using a titrator. The precipitates formed were oxidized using 1.7 M hydrogen peroxide (H₂O₂). 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 citric acid (CA). Second peptization was carried out for 3 hours using 1.25 M CA and finally washed with distilled water. The IONPs produced were collected and dispersed in water. The ferrofluids produced were characterized using X-ray diffractometer (XRD) (P8Advant-Bruker with Cu-Kα radiation source) to determine phases presence in the sample. Transmission electron microscopy (TEM) (Philips CM12.Version 3.2) was used to determine the size and distribution of IONPs and the concentration of Fe was determined using Beer’s law from UV–Visible near-infrared spectrophotometer (UV–Vis) (UV-3600, Shimadzu).

**Fabrication Nafion/GOx/CA-IONPs/ITO Biosensor.**

ITO glasses were cut and cleaned using alkaline Radio Corporation America (RCA) to improve the wettability. The ITO glass was immersed in ammonium hydroxide (NH₄OH), H₂O₂ 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. Then, 100 µL of various CA-IONPs concentration (1 mg/ml, 2 mg/ml, 3mg/ml and 4 mg/ml) was dropped cast on the ITO glass and dried in an oven at 80 °C for 2 hours. After that, 20 µL GOx (1 mg/ml) was immobilized and kept at 4 °C overnight. Finally, 20µL 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 by an Autolab Potentiostat/Galvanostat using three-electrode cell where ITO was 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.

**RESULTS AND DISCUSSION**

The typical XRD patterns of the samples were provided to characterize their specific structures (Figure 1). As can be seen, the diffraction peaks of the as synthesized IONPs and CA-IONPs are well indexed to the cubic structure of maghemite \( \gamma-Fe₂O₃ \) (JCPDS No.: 00-039-1346). This result was expected due to the oxidation process conducted using hydrogen peroxide (H₂O₂) 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 [15]. Extra vacancies in the \( \gamma-Fe₂O₃ \) structure are thought to be found in octahedral positions. No additional peaks have been observed, indicating the formation of a pure and single phase without impurities that remains from un-reacted precursors.
Transmission electron microscopy (TEM) was employed to characterize the dispersity and particle size of the prepared samples. Figure 2 shows the TEM image of the CA-IONPs and their size distribution. The CA-IONPs produced showed slightly spherical in shape with mode particle size of ~19 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 [15]. 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. Furthermore, the IONPs distributed uniformly without obvious aggregations due to the citrate stabilized.

The enzymatic glucose biosensor was fabricated by adsorption of GOx onto a IONPs film. The IONPs was selected as the matrix for immobilizing enzyme because of large surface area which is helpful for adsorbing the GOx without loss of its biological activity and further facilitate the transfer of electron. In order to optimize the available matrix for GOx immobilization on the working electrode, the effect of CA-IONPs concentration drop casted on the ITO bioelectrode was studied. The IONPs concentration drop casted on the ITO bioelectrode were varied (1 mg/ml, 2 mg/ml, 3 mg/ml and 4 mg/ml). Figure 3 showed the CV response of Nafion/GOx/CA-IONPs/ITO bioelectrode in 0.1M PBS (pH7) with presence of 1mM glucose. As the IONPs concentration increased from 1 mg/ml to 2 mg/ml, the magnitude of the oxidation current increased. These happen because at higher concentration, more CA-IONPs surface areas were available for GOx to immobilize. The presence of more CA-IONPs also greatly improved the conductivity and the electron transfer ability of the film [16]. Thus, the electrocatalytic activity toward glucose of Nafion/GOx/CA-IONPs/ITO bioelectrode was greatly improved. However, as the CA-IONPs concentration further increased to 3 mg/ml and 4 mg/ml, the magnitude of the oxidation current decreased significantly. These results could be attributed to the less active surface area available for GOx immobilization. At higher concentration, too many CA-IONPs presence on the electrode will tend to agglomerate, therefore less GOx could be immobilized [17]. The optimum CA-IONPs concentration to develop good electrocatalytic response glucose biosensor was at 2 mg/ml.

Figure 3: (a) CV response of Nafion/GOx/CA-IONPs/ITO bioelectrode in 1mM glucose into PBS (pH 7.0) at scan rate of 100 mV/s
Figure 4 shows the CVs studies of Nafion/GOx/CA-IONPs/ITO bioelectrode in the absence (curve a) and presence of 1-12.0 mM (curve b-j) glucose in 0.1 M PBS buffer (pH 7) at a scan rate of 100 mV/s. Here, 2 mg/ml CA-IONPs concentration was drop casted on the bioelectrode. In the absence of glucose (curve a), typical oxidation and reduction peaks was observed. When 1 mM of glucose was injected into the electrolyte, a noticeable anodic peak (curve b) was observed at the potential of -0.17 V and cathodic peak (curve b) at -0.48 V. A gradual increase in the oxidation peak current was observed upon further addition of glucose which revealed the excellent electrocatalytic activity of the modified electrode towards GOx enzyme presence on the electrode in sensing of glucose. The mechanisms of electrochemical behavior of the Nafion/GOx/CA-IONPs/ITO bioelectrode are summarized as follow (1)–(3).

\[
\text{Glucose} + \text{GOx} \rightarrow \text{Gluconolactone} + \text{GOx(r)} \quad (1)
\]
\[
\text{GOx(r)} + \text{O}_2 \rightarrow \text{GOx} + \text{H}_2\text{O}_2 \quad (2)
\]
\[
\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad (3)
\]
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 increased linearly with the increase in the glucose concentration (Figure 4). It was revealed that Nafion/GOx/CA-IONPs/ITO bioelectrode (inset of Fig. 4) could be used to estimate glucose from 1.0–12.0 mM. The sensitivity of the Nafion/GOx/CA-IONPs/ITO bioelectrode calculated from the slope of curve was found to be 90.44 µAmM⁻¹cm⁻² with linear regression of 0.98. This result is comparable to other works, which commonly used composite to modify the glucose biosensor electrode [1,17].

CONCLUSIONS

In this work GOx enzyme, CA-IONPs and nafion was successfully modified the ITO bioelectrode for glucose sensing. The optimum CA-IONPs concentration to develop good electrocatalytic response glucose biosensor was at 2 mg/ml. The immobilized GOx displays 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 are required. Due to the convenient in term of preparation and good properties, the studies have a significant potential impact on the selection of biosensing materials and on the design of electrochemical biosensors.

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REFERENCES


