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Paper Based Glucose Biosensor Depending on SPCE Modified with Hemoglobin and Silver Nanoparticles

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Abstract

In this study, new strategy intended for rapid glucose detection utilizing disposable glucose oxidase (GOD) paper disk integrated with screen-printed carbon electrode (SPCE) modified with hemoglobin (Hb) and silver nanoparticles (AgNPs). The GOD adsorbed on the surface of paper disk and after drying it placed on the surface of the modified SPCE and 16μL of glucose solution were added for the testing. Different parameters such as applied potential, concentration of enzyme, pH, size of paper disk, and the volume of buffer were optimized to improve the efficiency of this glucose biosensor.

Key Words: *Hemoglobin, Silver Nanoparticles, Glucose Biosensor, SPCE*

Introduction

Glucose monitoring is a requisite tool in clinical trials where glucose concentration is a definitive indicator in type 1 and type 2 diabetes mellitus and other diseases such as endocrine metabolic disorders. Most patients with diabetic need to test their blood sugar periodically. Thus, great interest have been aroused from industrials and marketers people to develop glucose sensor with more time-saving, much reliable, easy, cost-effective and can be utilized in home-based care [(1)]. Paper-based biosensor has drown much interest in analytical and clinical chemistry due to its discerning properties; cheap, simplicity, presorted reagent and not required specialized user, which can be largely used in the diagnostic field. Moreover, GOD is widely used in practical applications for glucose analysis, where it catalyzes glucose oxidation according to the following equation

Where, the glucose level can monitored by the detection of H_2O_2 generation or O_2 consumption. Overwhelmingly, electrochemical glucose biosensor based on O_2 detection but the detection of H_2O_2 was found to be more sensitivity.

Several analytical techniques have been employed for H_2O_2 analysis, such as spectrometry, fluorimetry, titrimetry, chromatography and electrochemical technique. Among these techniques, because of their rapid response, low cost, selectivity, simplicity and high sensitivity; the electrochemical method have been extensively utilized for this purpose [(2)].

Recently, with the large development in nanomaterial's science, SPEs became more utilized in smart and modern biosensor applications to avoid some common problems of classical solid, in addition to it is characterized with; low cost, disposable, convenience, flexibility in design, easy to chemical modification and reduction of sample volume required [(3)]. Different modifiers have been added in to screen printing ink to progress the sensitivity and selectivity of SPEs such as enzymes, mediators and nanomaterials [(4)]. Hemoglobin (Hb) has considered a suitable model for H_2O_2 biosensor because hemoglobin could act as peroxidase-like to reduce H_2O_2, commercially available with moderate cost and excellent stability and it has a well-known structure [(5)]. Nanoparticles attracted specific interests in the field of enzyme immobilization, due to unique properties, such as high electrical conductivity, good chemical and thermal stability, and large surface area [(6)].

In this study, we developed highly sensitive and stable paper based glucose biosensor by of utilized the previously fabricated hydrogen peroxide biosensor based on Hb immobilized on SPCE modified with AgNPs, and integration with GOD paper disk. Where,

in this paper based biosensor, glucose concentration can electrochemically detected by using 16µL of sample. In addition, the analytical efficiency of present biosensor was evaluated in term of repeatability, stability, sensitivity, and reproducibility after optimization of applied potential, pH, concentration of enzyme, and volume of buffer as the important parameters for biosensor development.

Experimental part

Apparatus and measurement

A potentiostat /Bipotentiostat (type DY2300, Digi-Ivy, Austin, U.S.A) was used to carry out the cyclic voltammetry, linear sweep voltammetry and Amperometric measurements, a screen-printed carbon electrode (SPCE) from Digi –Ivy (USA) with three electrodes was employed, consists of carbon electrode as working and counter electrodes, while pseudo Ag/AgCl was acted as a reference electrode. All the electrochemical experiments were done at room temperature (25 °C). The pH of the buffers was carried out using a HANNA pH meter. In cyclic voltammetry (CV) and linear sweep voltammetry (LSV) measurement, 16µL of 0.1M PBS (pH7) was added onto the paper disk, with this volume a good contact was obtained between the paper disk and the modified-SPCE.

Reagents

Silver nanoparticles (0.02 mg/mL, suspension in aqueous buffer, 10nm), and Nafion (5%) were purchased from Sigma- Aldrich. Hb, and H_2O_2 from Sigma (USA), chitosan (CS) from shanghai Biochemical (China), Glucose oxidase (GOD, 40IU/mg) from Fluka. A stock solution of 0.2M Na_2HPO_4, NaH_2PO_4 was used to prepare phosphate buffer solution (PBS, 0.1M) at various volume ratios and the pH was adjusted with 0.1M phosphoric acid or sodium hydroxide. All chemicals were of analytical reagent grade, and were used without further purification. Double distilled water (DDW) was used in all experiments. The stock solutions were stored at 4°C for further analysis.

Preparation of GOD paper disc

Grade 1 Whitman filter paper was carefully cut into round sheet with about 1 cm diameter using a paper punch. Then, 6µL of GOD solution (180 IU/mL) was carefully spotted on the center of each paper disk and left to dry at 25°C. The paper disk was placed onto the surface of the modified-SPCE to completely cover the working, reference and counter electrodes. Furthermore , GOD and glucose solutions with different concentrations were prepared and diluted with PBS (pH7).

Results and Discussion

Electrochemical investigation of paper-based analytical devices

The CV of modified SPCE integrated with GOD-paper disk and blank paper disk were examined by sweept the potential in window of -0.6 V to +0.3 V at scan rate 0.1Vs^{-1} in PBS (pH 7) with and without 1mM glucose solution, as shown in Figure (1).

No change in the cathodic and anodic peak current of modified SPCE was observed when blank paper was used in the presence of 1 mM glucose solution, in comparison with CV in PBS (pH 7) without glucose solution. Which refers to no H_2O_2 was produced in the absence of GOD in the reaction medium; also, the same result was obtained when GOD-disk paper was used in the absence of glucose solution.

While, there was an increase in the cathodic peak current of modified SPCE when glucose solution was added to paper disk pre-loaded with GOD, which is due to oxidation process of glucose, where the produced H_2O_2 in this reaction can be detect by the present modified SPCE.

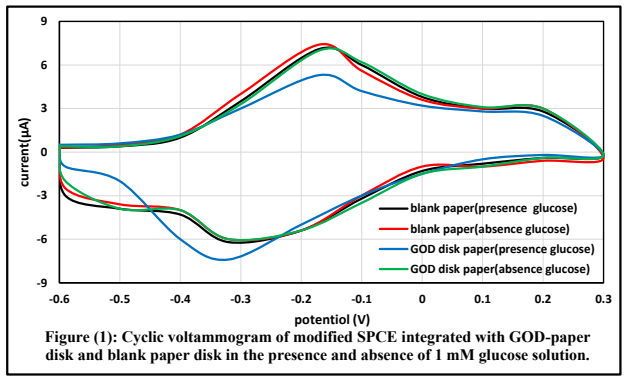

Figure (1): Cyclic voltammogram of modified SPCE integrated with GOD-paper disk and blank paper disk in the presence and absence of 1 mM glucose solution.

Direct electrochemistry behavior of GOD disk paper with modified SPCE was investigated in the detection of glucose level with different modified electrodes using LSV method in potential range from + 0.3 to -0.6 V at scan rate of 0.1Vs^{-1}. As shown in Figure (2), no reduction peak was observed for GOD-disk on the bare-SPCE in the presence of 1mM glucose solution, while, a weak peak current at -0.33 V was observed in Hb-

SPCE with GOD-disk paper. The reduction peak current was increased by 4.2 times when AgNPs was used in the modification of the electrode (NF-Hb-AgNP-CS-SPCE). Thus, GOD-disk paper integrated with NF-Hb-AgNP-CS-SPCE has a good electrocatalytic activity for oxidation of glucose, attributed to the high conductivity and high surface area of electrode that provided by using silver nanoparticles in modification of SPCE. In addition, it was demonstrated that Hb has a good electrocatalytic activity towards H_2O_2 reduction.

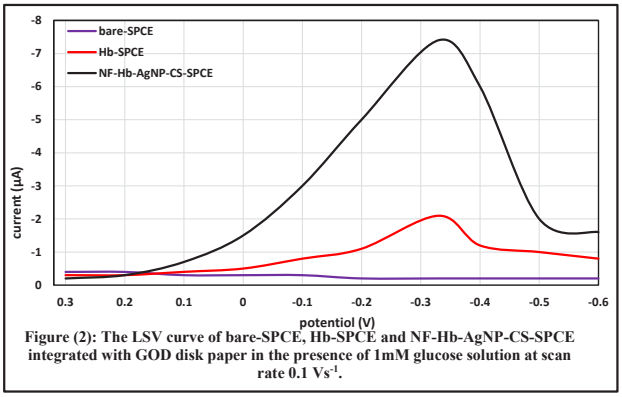

Figure (2): The LSV curve of bare-SPCE, Hb-SPCE and NF-Hb-AgNP-CS-SPCE integrated with GOD disk paper in the presence of 1mM glucose solution at scan rate 0.1 Vs^{-1}.

Optimization of operation conditions

The optimization of the GOD immobilization strategies in the fabrication of glucose biosensor is very important topic for obtaining high sensitive detection of glucose. The effect of applied potential on the sensing performance of fabricated glucose biosensor was firstly examined. Where, amperometry method is a simple, fast and very convenient technique for glucose quantitative determination. Thus, amperometric response of the present biosensor for 1 mM glucose solution were tested for 60 second at scan rate of 0.1 Vs^{-1} in different applied potential. It was found that the steady-state current increased as the detection potential increased from -0.2 to -0.33 V, which attributed to increase the driving force for fast reduction of H_2O_2. The response of paper-based glucose biosensor declined when the applied potential increase more than -0.33 V.

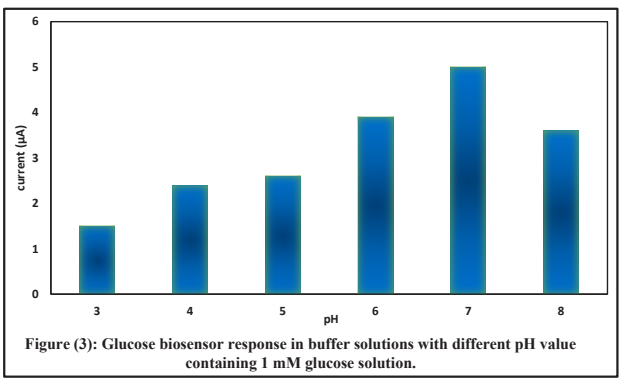

Figure (3): Glucose biosensor response in buffer solutions with different pH value containing 1 mM glucose solution.

When the amperometric response of the paper based analytical devices is typically affected by the enzyme loading [7]. Therefore, the amperometric experiment was investigated in 1 mM glucose solution for 60 s at scan rate of 0.1$Vs^{-1}$with several GOD-paper disks prepared by immobilized different concentration of enzyme 0.2 IU/disk - 1.2 IU/disk. The optimum sensitivity of biosensor was recorded with an enzyme concentration of 0.8 IU/disk. While, excess loading of the enzyme resulted unchanged in sensor response for glucose detection, which is identity with enzyme kinetics.

In this amperomtric study, PBS (pH 7) used as a carrier solution of analyte as well as supporting electrolyte. The volume effect of PBS containing constant concentration of glucose solution 0.1 mM on the amperometric response of GOD paper-based glucose biosensor was investigated in scan rate of 0.1 Vs^{-1}. Where, a suitable PBS volume was found to be 16 µL. In this volume of buffer, the contacting between the three electrodes of the modified SPCE and the wetting GOD paper was the best.

The pH value of the target buffer is a vital factor for the sensitivity of glucose biosensor, where two enzymatic reactions interested in the response of GOD paper-based NF-Hb-AgNP-CS-SPCE to glucose solution. An optimum pH rang influence both the electrochemistry of Hb and the bioactivity of the GOD. The extreme pH may change the kinetics of glucose measurements as a result of confusing the redox state of glucose oxidase reaction [8]. For this matter, the effect of pH on the amperometric response of paper-based glucose biosensor to 1 mM glucose solution was investigated in 0.1 M buffer solution between 3 to 8 pH. From Figure (3), it can be deduced that the optimum enzyme activity was found at (pH 7) and in pH value above or below that, there was clear drop in response of fabricated glucose biosensor that may be due to denaturation of enzyme in these pH value. Based on the above results, a concentration of GOD with 0.8 IU/disk, a potential of -0.33, 16 µL of PBS with pH 7 were selected to obtain the optimum sensing.

The characteristics of biosensor response

Amperometric method is very suitable method for determination of glucose concentration, since its utilization is characteristic with fast and simple. By employing the optimum experimental conditions as

described above, the calibration graph of the biosensor response to the concentration of glucose solution was shown in Figure (4). It can be seen that the present glucose biosensor enabled us to determine the glucose concentration from 1.2 mM to 5.2 mM, with regression equation

$$Y = 0.9059\,X + 3.0631$$

Where Y refer to the response (current) in μA and X refer to the concentration of glucose in mM, with a correlation coefficient of 0.9905.

The limit of detection for glucose was calculated as 0.6 mM (based on LOD =) and the sensitivity of biosensor was found to be 11.91 $\mu A.mM^{-1}.cm^{-2}$. The LOD value obtained was below 0.86 mM in Rungsawang, et al. study [9] and 3.12 mM in Li, et al. study [10]. Meanwhile, the sensitivity is higher than the previously reported glucose biosensor [11]. In addition , the resulted linear detection range of glucose that obtained by GOD paper disk integrated with NF-Hb-AgNP-CS-SPCE was 1.2-5.2 mM was almost the same as in other glucose biosensor , such as up to 5.7 mM in GOD-Au electrode [12], up to 6mM in GOD-AuNPs-NF-GC [13].

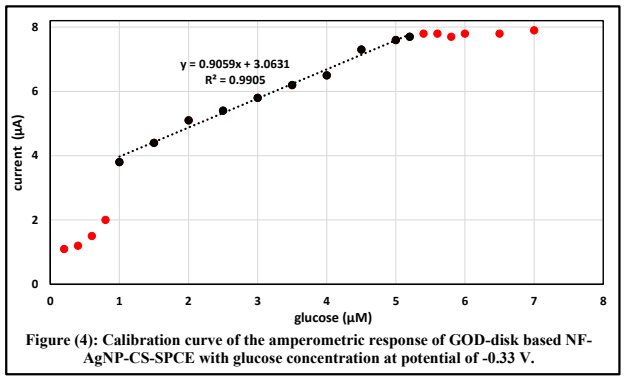

Figure (4): Calibration curve of the amperometric response of GOD-disk based NF-AgNP-CS-SPCE with glucose concentration at potential of -0.33 V.

a substrate saturation was observed when the glucose concentration is over than (5.2 mM), showing the characteristics of Michaelis-Menten kinetic (typical nzymatic reaction kinetics). The value of the apparent Michaelis-Menten constant () was calculated to be (4.4 mM), this value was significantly lower than 35mM in GOD-AuNPs-PA [14]. Which implying that the GOD in our experiment exhibits a remarkable affinity for glucose.

Stability and reproducibility of glucose biosensor

In this work, the reproducibility of the biosensor was monitored by using 10 GOD paper disks prepared independently for testing 2 mM glucose solution, R.S.D was calculated to be 3.53%, while the bio electrocatalytic signal reached 90% of the steady-state value in 15 s.

The stability of the newly prepared GOD paper disks was evaluated at optimum conditions, by monitoring the signal decrease with storage time. The response of GOD paper based NF-Hb-AgNP-CS-SPCE was decreased to 62% of initial response after (20 days) of storage at 4°C. Thus, in this work the designed GOD paper based glucose biosensor can detect the concentration of glucose in small volume of sample with high sensitivity, good stability and low cost technique.

Selectivity of biosensor.

Selectivity of the glucose biosensor is important feature for practical applications, where in the amperometric biosensor, the electroactive compounds have been a problem in testing the biological or industrial samples. Eight possible potential interference species that may be influences the amperometric response of the proposed glucose biosensor were studied. The paper-based glucose biosensor response for a fixed concentration of glucose 2 mM was compared to that obtained for 1 mM of the possible interfering compound in the presence and absence of 2 mM glucose solution. As can be seen in Table (1), the paper-based glucose biosensor does not give response to any one of the interference compound that studied in the absence of glucose solution (close to background), that due to the specificity of enzyme.

However, in the presence of 2mM glucose solution plus fixed amounts of interfering species 1mM, the amperometric response is changes lower than 7% with exception of cysteine, where the registered signal decreased 12.3% that may refer to its interaction with some amino acid resides in the glucose oxidase molecule. Hence, our fabricated paper-based glucose biosensor are very suitable for the selective glucose determination in biological or industrial samples.

Table (1): Study of the interferences caused by different compounds to the response of paper-based glucose biosensor.

Sample without glucose	Response (μA)		Sample with glucose	Response (μA)
Cysteine	0.83		Cysteine-glucose	4.56
Fructose	0.74		Fructose-glucose	5.1
Vit.C	0.81		Vit.C-glucose	5.05
Urea	0.45		Urea-glucose	5.2
Uric acid	0.67		Uric acid-glucose	4.96
Sucrose	0.71		Sucrose-glucose	4.86
Xylose	0.86		Xylose-glucose	4.91
Ca^{++}	0.64		Ca^{++}	5.06
Mannose	0.52		Mannose-glucose	5.12
Background (PBS, pH7)	0.35		(2mM) Glucose	5.20

(n=3) SD= standard deviation

Conclusion

The paper-based biosensor comprised a linear response range for glucose over 1.2 mM to 5.2 mM with detection limit of 0.6 mM at signal to noise ratio of 3. While, the sensitivity and the apparent Michaels-Menten constant () were calculated and found to be 11.91 $\mu A.mM^{-1}.cm^{-2}$ and 4.4 mM. In addition, the stability, reproducibility, repeatability and selectivity of present glucose biosensor were monitored in this study

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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