Research Article

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Development of a highly sensitive glucose nanocomposite biosensor based on recombinant enzyme from corn

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Abstract

BACKGROUND: Enzymes are biocatalysts that play a vital role in the production of biomolecules. Plants can be a valuable and cost-effective source for producing well-structured recombinant enzymes. Glucose is one of the most important biological molecules, providing energy to most living systems. An electrochemical method for immobilization of enzyme is promising because it is economic, generates less component waste, improves the signal-to-noise ratio, leads to a lower limit of detection, and stabilizes and protects the enzyme structure.

RESULTS: A glucose biosensor was constructed using polyaniline (PANI) and a recombinant enzyme from corn, plant-produced manganese peroxidase (PPMP), with polymerization of aniline as a monomer in the presence of gold nanoparticles (AuNPs)-glucose oxidase (GOx), and bovine serum albumin. Using linear sweep voltammetry and cyclic voltammetry techniques, PANI-AuNPs-GOx-PPMP/Au electrode exhibited a superior sensing property with a wider linear range of 0.005–16.0 mM, and a lower detection limit of 0.001 mM compared to PANI-GOx-PPMP/Au electrode and PANI-GOx-PPMP/AuNPs/Au electrode. The biosensor selectivity was assessed by determining glucose concentrations in the presence of ascorbic acid, dopamine, aspartame, and caffeine.

CONCLUSION: We conclude that a plant-produced Mn peroxidase enzyme combined with conductive polymers and AuNPs results in a promising nanocomposite biosensor for detecting glucose. The use of such devices for quality control in the food industry can have a significant economic impact.

Supporting information may be found in the online version of this article.

Keywords: recombinant enzyme from corn; electrochemical biosensors; gold nanoparticles; polyaniline; glucose

INTRODUCTION

Enzymes play a key role in improving various products' physical, chemical, and sensory properties. Recently we applied a recombinant enzyme from corn kernels to fabricate novel electrochemical-based biosensors for glucose and hydrogen peroxide determination. Chronoamperometric and cyclic voltammetry results showed a great potential of plant-produced manganese peroxidase (PPMP) to meet the growing demands for enzymes in commercial applications.[1,2]

Glucose metabolism occasionally becomes uncontrolled causing a myriad of health problems. Worldwide, the number of people with diabetes is currently estimated by the International Diabetes Federation at around 500 million,[3] and this number is steadily increasing, which puts a heavy burden on the health care system. Therefore, although there are multiple glucose biosensors available on the market, there is still a solid need to lower their cost and enhance their sensitivity, selectivity, stability, simplicity, and accessibility to users. Also, close monitoring of glucose levels in biological samples is essential to ensure that a low amount is present.[4-6] Traditional glucose sensors are enzymatic in nature, mainly using glucose oxidase enzyme in combination with enzymes from fungi, for example, the industrially accepted *Aspergillus niger* enzyme, which is expensive and has low activity.[7,8]

Our previous work showed that PPMP has excellent activity[1,2] and we fabricated a simple and inexpensive biosensor using this novel enzyme and polymeric nanocomposites. Mechanical strength and low reactivity to aging agents are always required for sensor coatings. Because of this, polymeric nanocomposites, which offer strength and low reactivity, play an essential role in

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measuring, processing, and activating electrodes for electrochemical and biosensing applications. To take full advantage of these compositions, they must have good processing capability, which is ultimately driven by the use of conductive polymers (CPs) as one component of the nanocomposites.[9] π–π conjugated polymers, which can be synthesized via chemical or electrochemical oxidation of monomers, are used to develop rechargeable batteries and sensors.[10-13] These CPs are characterized by excellent electrical properties, are light weight, efficient, low cost, flexible, have high biocompatibility and act as redox mediators to facilitate the transfer of electrons to the sensor.[14] Polyaniline (PANI) as an CP is one of the most commonly studied and utilized conducting polymers because of its easy and inexpensive synthesis: by oxidation of aniline.[15-17] Although PANI is pseudocapacitive, it still suffers from limited cycling stability and high auto-discharge.[18] To solve this limitation, PANI can be combined with various mineral nanomaterials such as gold nanoparticles (AuNPs) as composites.[19-21]

The properties of nanocomposites depend on the shape and volume fraction, the morphology, and the nature of the interphase between the two components.[22] AuNPs enhance the sensitivity of a sensor and have many advantages such as a large specific area, biocompatibility, and high chemical stability.[23-26]

Immobilizing enzymes on an electrode is a well-known method to improve enzyme reusability and stability and enhance the capacity and accessibility of biomolecules.[27,28] Immobilization of the target enzyme on conductive nanomaterials facilitates electron transfer in the sensor system, because enzymes by themselves are not generally conductive The synthesis performance using an electrochemical technique is fast and easy by anodic oxidation of the monomer onto working electrodes.[29,30] In addition, variability of membrane thickness is achieved by adjusting the applied potential during electropolymerization.[2,31-33]

A literature review shows that horseradish peroxidase with glucose oxidase (GOx) is the most common enzyme pair for glucose detection. Horseradish peroxidase has several disadvantages for glucose detection, including high cost and finite reaction conditions. In the present study, we used recombinant Mn peroxidase from corn (i.e. PPMP) to detect glucose to solve these problems. PPMP is a recombinant fungal manganese peroxidase[1,2] that is produced in a cost-effective plant production system. The enzyme gene (Genbank access #: 129039) was cloned from the white-rot fungus *Phanerochaete chrysosporium* and expressed recombinantly in corn kernels.[34]

In the present study, we co-immobilized PPMP, AuNPs, and GOx onto a gold electrode by electropolymerization of PANI to demonstrate a stable, sensitive, selective, low-cost, and flexible glucose sensor. Experiments were then performed to evaluate the effect of substrate flexibility on the performance of the modified electrode, and the PANI film deposition was the only critical parameter. The sensing ability of the thin films with respect to glucose was evaluated by linear sweep voltammetry (LSV) and cyclic voltammetry (CV), which show good controllability and reproducibility of this glucose sensor. Characterization of the biosensor membrane was studied by scanning electron microscopy (SEM), which revealed different morphologies of the various composites. Finally, the selectivity and applicability of glucose detection using ascorbic acid (AA), dopamine (DA), aspartame, and caffeine were investigated.

MATERIALS AND METHODS

The glucose stock solution was prepared by mixing a given amount of α-D-glucose anhydrous, 96% from Sigma-Aldrich (St Louis, MO, USA) in phosphate buffer (NaPB; pH 7.0). The prepared stock was diluted to the desired concentrations. Manganese (II) acetate tetrahydrate 9.99% [Mn $(CH_3COO)_2$], bovine serum albumin (BSA), glucose oxidase from *Aspergillus niger*, phosphate buffer solution (PBS) using NaH_2PO_4, and Na_2HPO_4, gold nanoparticles (20 nm, suspension in 0.1 mM PBS), and aniline were purchased from Sigma-Aldrich. All of the solutions were prepared in Milli-Q deionized water (Merck Millipore, Burlington, MA, USA) (18.2 MΩ) unless otherwise noted. All of the experiments were conducted at laboratory room temperature (20°C).

Electrode construction

Modified electrodes were prepared by immobilizing PPMP and GOx by electropolymerizing PANI onto a 5-mm diameter polished gold electrode.[1] A mixer was provided including 0.5 M PPMP, 0.17 M aniline, 0.10 M GOx, and 2.4 μM BSA in PBS (pH 7.0), and then the Au electrode was immersed into this composite. Polymerizations were conducted by the cyclic voltammetric sweep of 10–25 cycles with potential ranging from −0.25 to 0.45 V/Ag/AgCl, at a scan rate of 0.05 Vs^{-1}. Polymer nanocomposites show the best stability using 20 cycles used in all of our electrode modifications.

Apparatus

All voltammetry and amperometric measurements were performed using a computer-controlled CHI660D electrochemical workstation (CH Instruments, Austin, TX, USA). Experiments were carried out in a three-electrode cell with a platinum wire (0.25 mm in diameter, 99.9%; Alfa Aesar, Ward Hill, MA, USA) as a counter electrode and a 5-mm diameter gold electrode as the working electrode, and Ag/AgCl (3 M KCl) as the reference electrode as follows:

Ag/AgCl || xM (glucose) (aq), 0.1 M phosphate buffer (pH 7.0) and 0.1 mM ($MnSO_4$) | polymer matrix [(PPMP (0.5 M)-aniline(0.17 M)-GOx (0.10 M)-BSA (2.4×10^{-6} M)-AuNPs(1.0 g)] | Au Cell (1).

A NANO 1000S single wheel grinder timer bench top polisher with variable speed 100–1000 rpm, rapid programmable speed and time selection from Pace Technologies Corporation (Tucson, AR, USA) was applied to polish the gold electrode surface. A benchtop vacuum spin coater (8000 rpm) from MTI Corporation (Richmond, CA, USA) was applied for electrode spin coating. SEM images were recorded on an SNE-4500M Plus Tabletop Scanning Electron Microscope (Nano Images, LLC, Pleasanton, CA, USA).

RESULTS AND DISCUSSION

Linear sweep voltammetry performance of the PANI-GOx-PPMP/au electrode

The electropolymerizing of the PANI-GOx-PPMP/Au electrode is characterized by an increase in the anode current corresponding to the oxidation of the aniline monomer to form the polymer and a reduction peak current is observed, attributed to the reduction of the aniline formed during its oxidation. There are two peaks observed in the oxidation and reduction sweep. The anodic peak was observed at −106 and +119 mV, and the cathodic peak was found at −102 and 25 mV. The oxidation peak at −106 mV is polyaniline, which is oxidized into a semi-oxidized stat. In comparison, +119 mV is the oxidation peak of the semi-oxidized state, which is oxidized into the fully oxidized form of polyaniline (Fig. S1). The modified electrodes were stored in an incubator at 4°C when not in use. The process for fabricating the PANI-GOx-PPMP/Au modified electrode is schematically shown in Fig. 1(A).

To monitor the characteristics of the modified electrode, we applied LSV using a three-electrode system where a platinum wire was used as an auxiliary electrode, an Ag/AgCl electrode as a reference, and the PANI-GOx-PPMP/Au electrode as the working electrode. Figure 2(A) represents the LSV results of glucose oxidation on the PANI-GOx-PPMP/Au electrode. The LSV was conducted in a 10 mL of solution of PBS, pH 7, containing 0.1 mM $Mn(CH_3COO)_2$ in a potential range from 0.1 V to 1.1 V/Ag/AgCl, at a scan rate of 0.05 Vs^{-1} in an oxygen saturated solution. The detection of glucose was made indirectly by monitoring the oxidation of H_2O_2 because H_2O_2 was released with a reaction of glucose and oxygen in the presence of GOx, and H_2O_2 is reduced to water by the PPMP.

$$\text{Glucose} + O_2 \xrightarrow{GOX, PPMP} \text{Gluconolactone} + H_2O_2 \qquad (1)$$
$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e.$$

Upon addition of glucose, oxidation peaks increase proportionally at +0.80 V/Ag/AgCl, at a scan rate of 0.05 $V\,s^{-1}$. A sharp increase in current was associated with increasing glucose concentration and peak potential shift to more positive, indicating an increase in the local concentration of oxygen on the surface of the PANI-GOx-PPMP/Au electrode. Figure 2(B) shows the calibration plot of the response current to different glucose concentrations of the PANI-GOx-PPMP/Au electrode. Good linear response with increased glucose concentration was obtained within 0.05–15.0 mM with a limit of detection of 0.016 mM. The regression line (R^2) values, which fitted well to the calibration curves, were 0.9991. The limit of detection (LOD) was calculated for $S/N = 3$ and $S = (3 \times \text{standard deviation}) + \text{blank}$.[35] Standard deviation, S, is calculated as shown by Eqn (1) (N is the number of standard solutions). $N - 2$ is the degree of freedom.

$$S = \sqrt{\frac{\sum y_i^2 - \frac{(\sum y_i)^2}{N} - m^2\left[\sum x_i^2 - \frac{(\sum x_i)^2}{N}\right]}{N-2}} \qquad (2)$$

The data indicated an obvious electrocatalytic response of PANI-GOx-PPMP/Au electrode and can be used to quantitatively monitor glucose.

Linear sweep voltammetry performance of the PANI- GOx-PPMP/AuNPs/au electrode

To explore the effect of AuNPs on biosensor performance, we prepared a new PPMP enzyme biosensor based on gold nanoparticles that were directly spin-coated onto the Au electrode. The electrode was stirred at 500 rpm for 5 min using the spin coater. Three AuNPs/Au electrodes were prepared with different nanoparticle coverage by controlling the amount of deposition, two of 7 µL, two of 10 µL, and two of 15 µL. In the first stage of coverage, the 7 µL of AuNPs solution composition, the second 7 µL treatment of AuNPs was dropped 10 min later into the same location, and the same method was performed to apply other amounts. Afterward, a solution containing a mixture of 0.5 M PPMP, 0.17 M aniline, 0.10 M GOx, and 2.4 µM BSA in PBS (pH 7.0) was prepared. The AuNP coated electrode was immersed into the enzyme solution and, similar to that already described above, the composite was immobilized with a continuous cyclic voltammetric of 20 cycles on the AuNPs/Au. This electrode was called PANI-GOx- PPMP/AuNPs/Au, and the prepared electrodes were stored in an incubator at 4°C when not in use. Electrochemical measurements were carried out in a cell containing 10 mL of 0.1 M PBS (pH 7.4) containing 0.1 mM $Mn(CH_3COO)_2$ at a scan rate of 0.05 Vs^{-1}. We performed LSV on the PANI-GOx-PPMP/AuNPs/Au electrode as the working electrode in the three-electrode system as described above. The best sensor response was from the

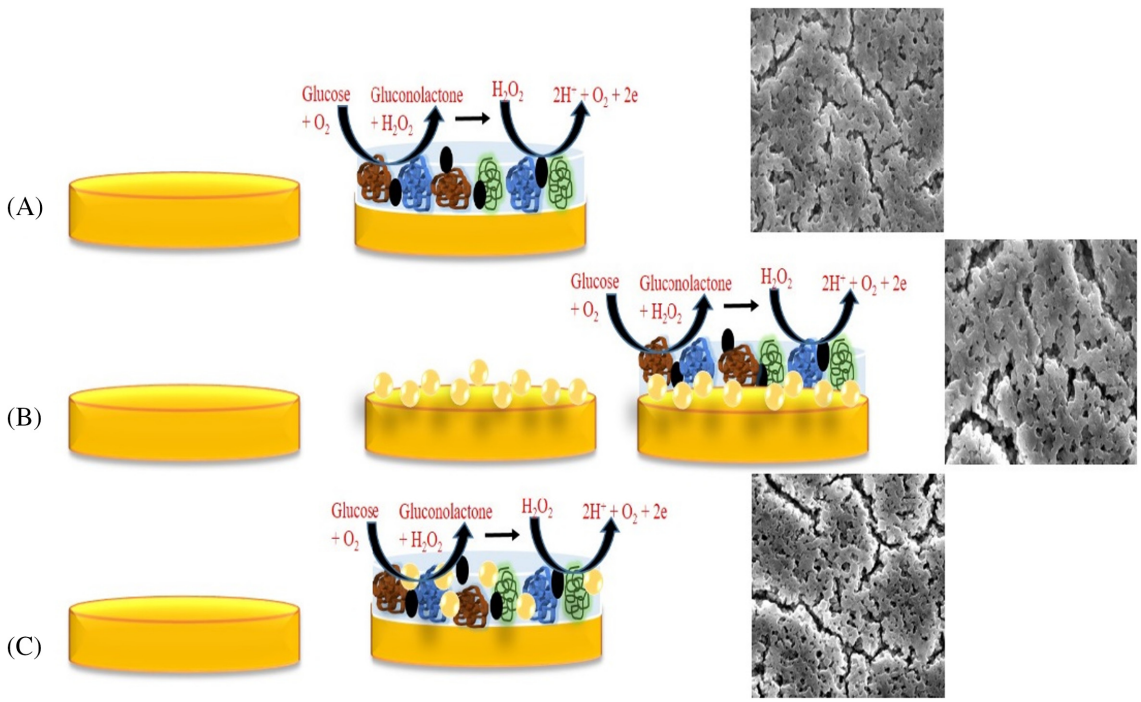

Figure 1. (A) Schematic illustration of the electrochemical modification of the PANI-GOx-PPMP/Au electrode and its SEM image. (B) Schematic illustration of the electrochemical modification of the PANI-GOx-PPMP/AuNPs/Au electrode and its SEM image. (C) Schematic illustration of the electrochemical modification of the PANI-GOx-PPMP-AuNPs/Au electrode and its SEM image.

electrode that was spin-coated with two doses of 7 µL of AuNPs. As shown in Fig. 3(A), in the LSV of the PANI-GOx-PPMP/AuNPs/Au modified electrode, the anodic peak current at 0.83 V correlated well with the increased glucose concentrations. The PANI-GOx-PPMP/AuNPs/Au electrode has a detection limit of 0.003 mM with a linear range of 0.010–15.0 mM (Fig. 3B). The R^2 values of the regression lines that fitted to the calibration curves were 0.9991. The results show that the PANI-GOx-PPMP/AuNPs/Au electrode shows greater electrocatalytic activity toward glucose determination than the PANI-GOx-PPMP/Au electrode. More activity and lower LOD are the result of an increase in the electrode's conductivity and larger surface area, which exhibits better electron transfer. Furthermore, the higher peak currents of the PANI-GOx-PPMP/AuNPs/Au electrode compared to the PANI-GOx-PPMP/Au electrode exhibits the most effective electron transfer, which confirms that AuNPs are a great platform for increasing enzyme load and effectively improving charge transfer between the glucose and the electrode surface. Peak potential is slightly higher for the PANI-GOx-PPMP/AuNPs/Au electrode than the PANI-GOx- PPMP/AuNPs/Au electrode, which could be the result of a change in the mechanism of electron transfer using gold nanoparticles.

Linear sweep voltammetry and cyclic voltammetry performance of the PANI-GOx-PPMP-AuNPs/au electrode

We applied AuNPs *in situ* directly to the PANI matrix. A 5-mm diameter polished Au electrode was immersed in a solution containing 0.5 M PPMP, 0.17 M aniline, 0.10 M GOx, 2.4 µM BSA, and

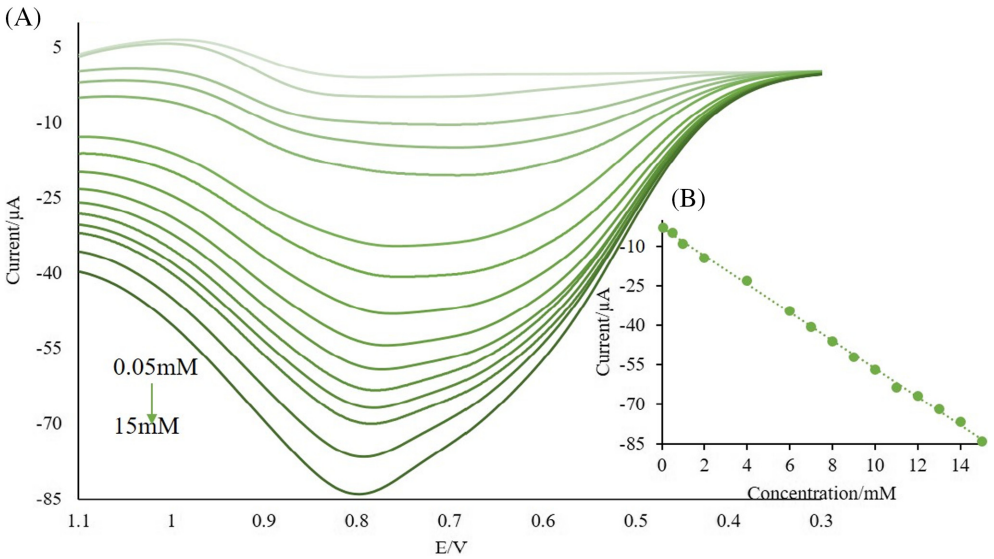

Figure 2. (A) Background-subtracted of LSVs for a PANI-GOx-PPMP/Au electrode, in the oxygen saturated PBS/0.1 mM $Mn(CH_3COO)_2$ solution in the range 0.05–15.0 mM glucose *versus* Ag/AgCl (scan rate of 0.05 V s^{-1}). (B) Calibration plot of background-subtracted peak current *versus* glucose concentration (R^2 = 0.9991).

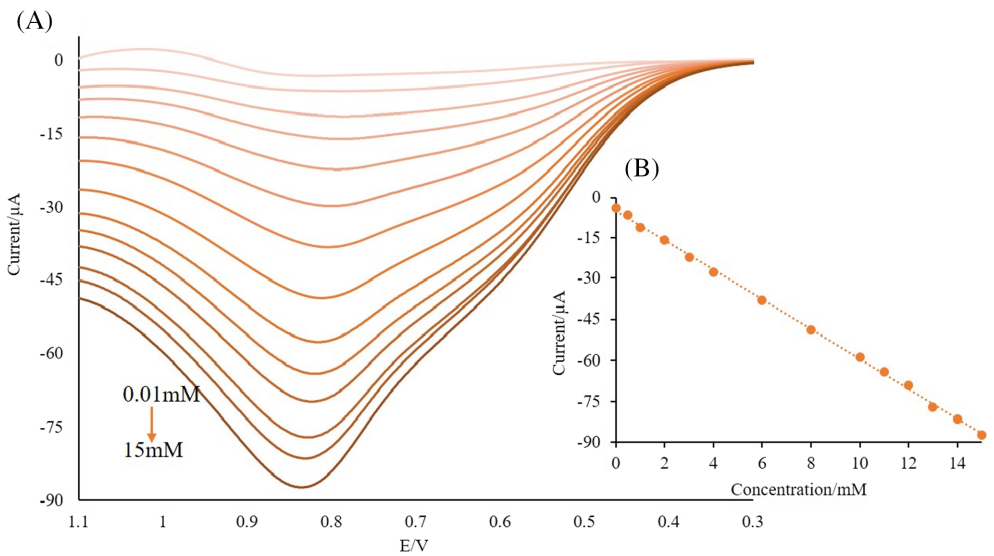

Figure 3. (A) Background-subtracted of LSVs for a PANI-GOx-PPMP/AuNPs/Au electrode, in the oxygen saturated PBS/0.1 mM $Mn(CH_3COO)_2$ solution in the range 0.01–15.0 mM glucose *versus* Ag/AgCl (scan rate of 0.05 V s^{-1}). (B) Calibration plot of background-subtracted peak current *versus* glucose concentration (R^2 = 0.9991).

1.0 g of AuNPs in PBS (pH 7.0) and electropolymerized as above. The electrode was called PANI-AuNPs-GOx-PPMP/Au, and the prepared electrodes were stored in an incubator at 4°C when not in use. In the typical cell setup described above, the PANI-AuNPs-GOx-PPMP/Au electrode was used as the working electrode, a platinum wire was used as an auxiliary electrode, and an Ag/AgCl electrode was the reference. Electrochemical measurement was carried out by LSV as above to characterize the electrochemical behaviors of the PANI-AuNPs-GOx-PPMP/Au electrode. The LSV results (Fig. 4A) show that the oxidation peak of AuNPs is at +0.94 V at a scan rate of 0.05 Vs^{-1}. A good linear response with increased glucose concentration was obtained within a wide range of 0.005–16.0 mM. The R^2 values of the regression lines that fitted to the calibration curves were 0.9990 with a lower limit of detection of 0.001 mM (Fig. 4B). The PANI- GOx-PPMP-AuNPs/Au electrode exhibits a more comprehensive linear range and a lower detection limit compared to the PANI-GOx-PPMP/AuNPs/Au electrode and the PANI-GOx- PPMP/Au electrode. We observed (Fig. 4A) a higher peak current for this modified electrode because of the conducting nature of AuNPs and the increased active surface area of nanocomposites, which offers more freedom and activity of enzymes.[36] The results confirm that the larger surface area facilitates the high enzyme loading and lower LOD of PANI-GOx- PPMP-AuNPs/Au electrode compared to the

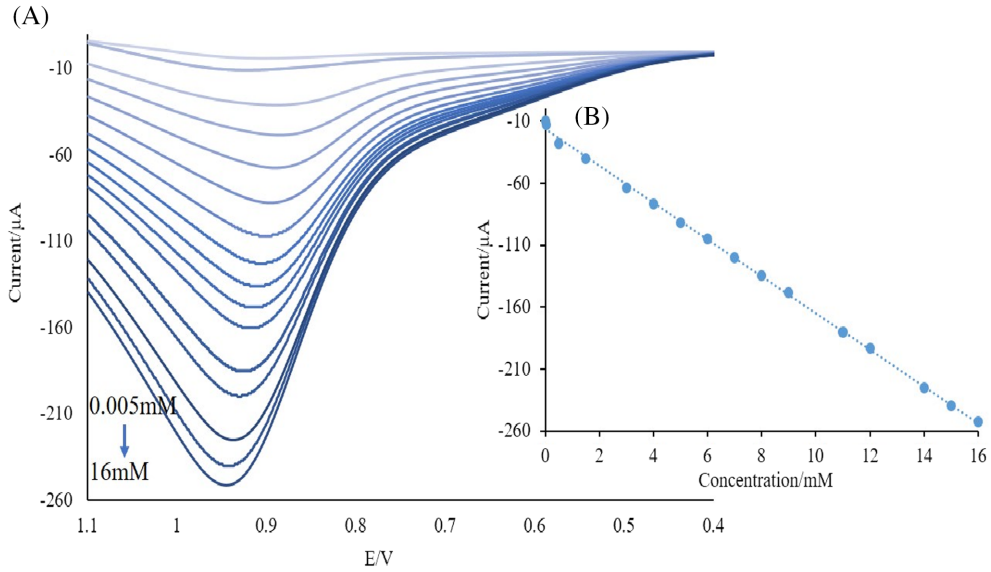

Figure 4. (A) Background-subtracted of LSVs for a PANI-GOx-PPMP-AuNPs/Au electrode, in the oxygen saturated PBS/0.1 mM $Mn(CH_3COO)_2$ solution in the range 0.05–16.0 mM glucose *versus* Ag/AgCl (scan rate of 0.05 V s^{-1}). (B) Calibration plot of background-subtracted peak current *versus* glucose concentration (R^2 = 0.9990).

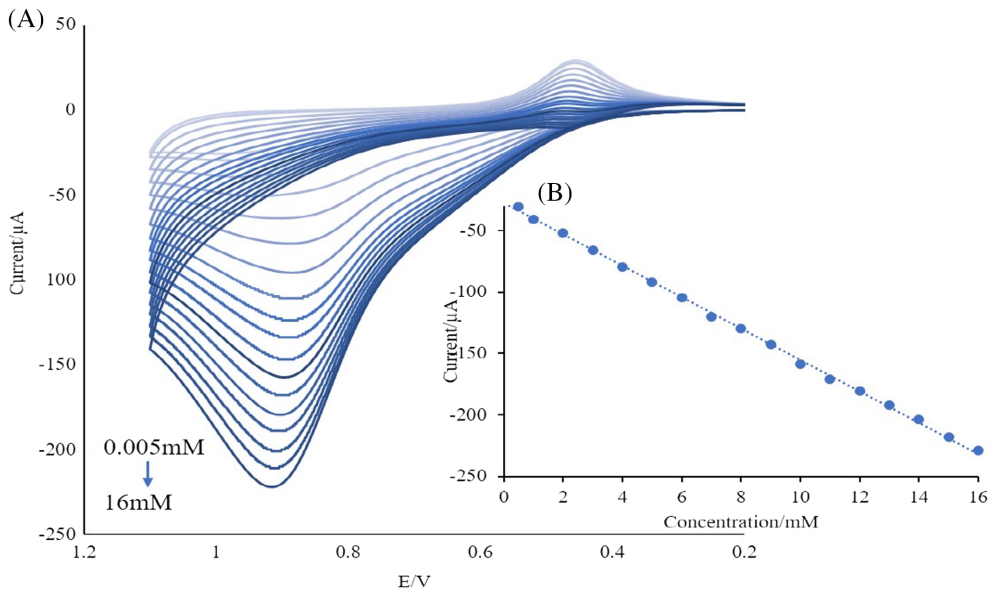

Figure 5. (A) Background-subtracted of CVs for a PANI-GOx-PPMP-AuNPs/Au electrode, in the oxygen saturated PBS/0.1 mM $Mn(CH_3COO)_2$ solution in the range 0.05–16.0 mM glucose *versus* Ag/AgCl (scan rate of 0.05 V s^{-1}). (B) Calibration plot of background-subtracted peak current *versus* glucose concentration (R^2 = 0.9991).

PANI-GOx- PPMP/AuNPs/Au electrode. Moreover, the PANI- GOx-PPMP-AuNPs/Au electrode, as a result of π–π^* benzene ring transfer of polyaniline in nanocomposites, shows incremental electrical conductivity increases.

The sensing capability of PANI-GOx- PPMP-AuNPs/Au electrode nanocomposites towards glucose detection was also investigated by CV in the same cell setup. Figure 5(A) indicates that the oxidation peak at +0.91 V at a scan rate of 0.05 V s^{-1} increased significantly with the addition of glucose, indicating the responsivity of the biosensor toward glucose. Furthermore, the gold reduction peak in a cathodic scan appeared at 0.44 V. The change of peak potential of CV (0.91 V) compared to LSV (0.94 V) to a slightly lower voltage value is probably the result of changes in AuNPs-mediated electron transfer kinetics. Proportional increases of oxidation peaks with glucose addition were also observed (Fig. 5B). An increase in glucose concentration correlated with dramatic increases in current and shifted the oxidation peak towards more a positive potential, which indicated a rise in the local concentration of oxygen on the PANI- GOx-PPMP-AuNPs/Au electrode surface. The calibration plot of the current–concentration response curve showed that it has a linear relationship with a correlation coefficient of 0.9991. The designed biosensor possessed a excellent linear response between 0.005–16.0 mM. The LOD of 0.001 mM is much lower than that in our previous work with the

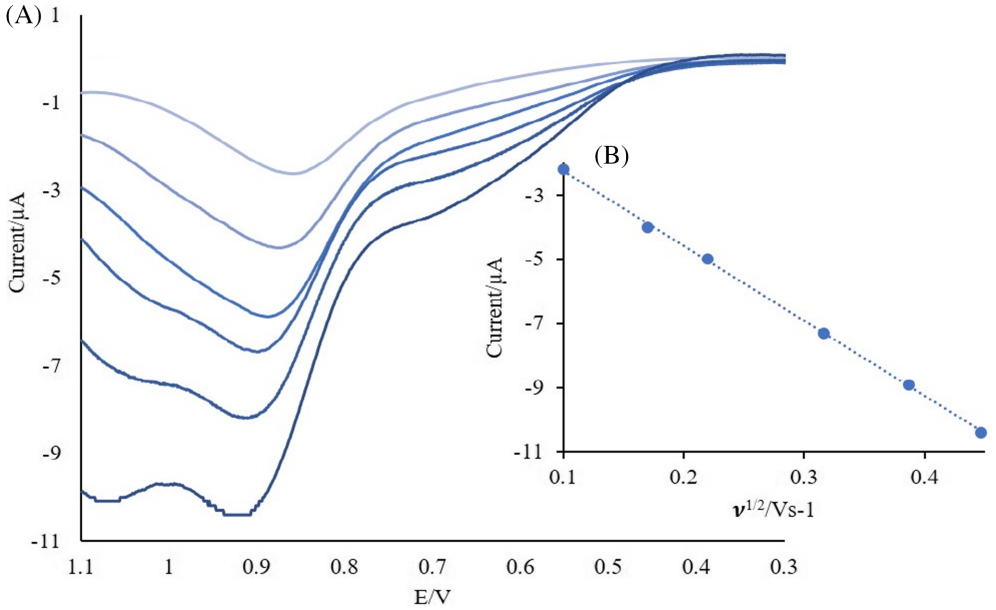

Figure 6. (A) Background-subtracted LSV plots of the PANI-GOx-PPMP-AuNPs/Au electrode, at different scan rates of 0.01, 0.03, 0.05, 0.07, 0.10, and 0.20 V s^{-1} *versus* Ag/AgCl in 0.050 mM glucose in PBS pH 7/0.1 mM $Mn(CH_3COO)_2$ in an oxygen saturated solution. (B) Linear relationship between the oxidation peak current and the square root of the scan rate (R^2 = 0.9995).

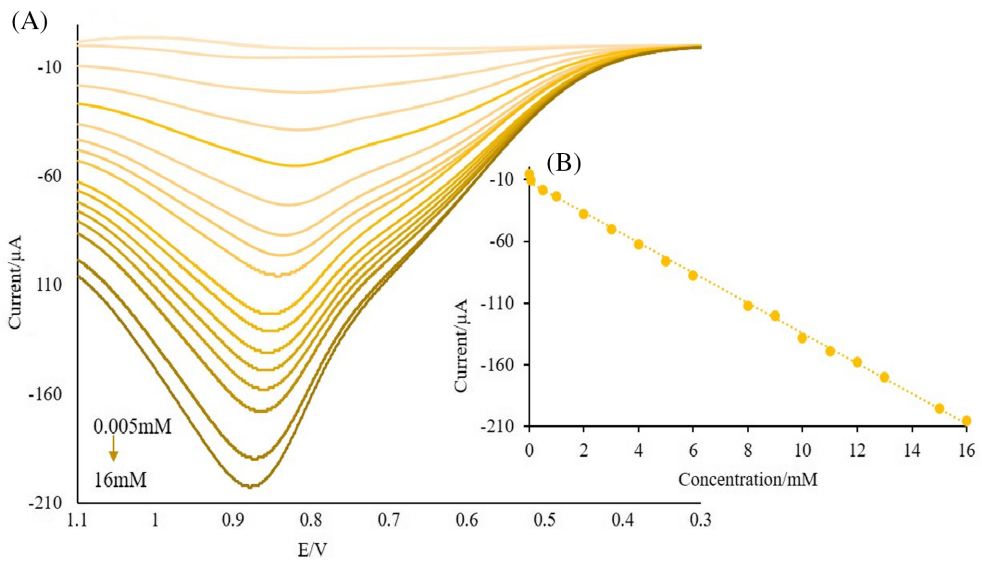

Figure 7. (A) Background-subtracted LSVs plots of PANI-GOx-PPMP-AuNPs/Au electrode, in the oxygen saturated PBS/0.1 mM $Mn(CH_3COO)_2$ solution containing DA in the range 0.005–16.0 mM glucose *versus* Ag/AgCl (scan rate of 0.05 V s^{-1}). (B) Calibration plot of background-subtracted peak current *versus* glucose concentration (R^n = 0.9985).

Nafion/PPMP-GOx-PBS/Au electrode,[2] as well as other published work such as with a PtCo/NPG/GP electrode,[37] a GR/PANI-AuNPs (6 nm)-GOx/GOx electrode,[38] and a $AuNP_s$-decorated MoS_2 nanocomposite electrode.[39]

Responses of the PANI-GOx-PPMP-AuNPs/au electrode at different scan rates

To further characterize our newly constructed electrode, the typical diffusion-controlled electrochemical behavior was also established by the background-subtracted LSV response of the PANI-GOx-PPMP-AuNPs/Au electrode at different scan rates in the same cell set up (Fig. 6A). The sweep rate was changed from 0.01 to 0.20 Vs^{-1} in 0.050 mM glucose in PBS pH 7/0.1 mM $Mn(CH_3COO)_2$ in an oxygen saturated solution. According to the Randles Sevcik equation[40]:

$$i_p = \left(2.687\times10^5\right)n^{\frac{3}{2}}A.C(D.\nu)^{1/2}$$

where i_p is peak current, n is the number of electrons transferred, A is the electrode surface area, C is the molar concentration, D is the diffusion coefficient, and ν is the scan rate in Vs^{-1}, the oxidation peak current was proportional to the square root of the scan rates linear relationship with a correlation coefficient of 0.9995 (Fig. 6B). This linear relationship indicates that the electrochemical process was controlled by diffusion and verifies that the electron transfer processes involve freely diffusing glucose. Moreover, there is no significant change in the peak potential, which shows the linear behavior of current as a function of the square root of scan rates.

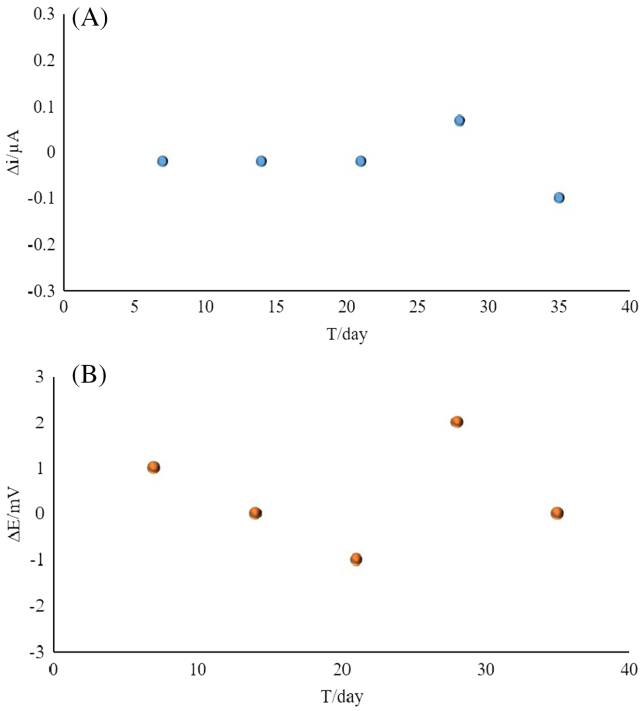

Figure 8. (A) Storage stability of current of the PANI-GOx-PPMP-AuNPs/Au biosensor in PBS (pH 7) at a glucose concentration of 300 μM. (B) Storage stability of potential of the PANI-GOx-PPMP-AuNPs/Au biosensor in PBS (pH 7) at a glucose concentration of 300 μM.

Selectivity, stability, and reproducibility of the PANI-AuNPs-GOx-PPMP/au biosensor

An important characteristic of glucose biosensors is the selective response in the presence of other interfering species. The selectivity of the biosensor was examined in the presence of 0.10 mM AA, DA, Aspartame, and Caffeine. The effect was tested by adding these interferences during glucose measurement using LSV. It was observed that these compounds had no interfering effects on the analysis of glucose. Figure 7(A) shows the selectivity of the biosensor in the presence of 0.10 mM DA. A good linear response was obtained within 0.005–16.0 mM (R^2 = 0.9985) with a limit of detection of 0.001 mM (Fig. 7B). A clear current response was observed with the addition of glucose, and negligible effects on the current response were observed when 0.10 mM of AA, aspartame, or caffeine were added into the solution (Figs S2–S4).

The stability of the PANI-GOx-PPMP-AuNPs/Au glucose biosensor was determined by performing activity assays of PPMP on current within 35 days in 0.05 mM glucose (Fig. S5). The activity assay was applied within 35 days to determine the storage stability of the immobilized enzyme. As shown in Fig. 8(A), an activity loss

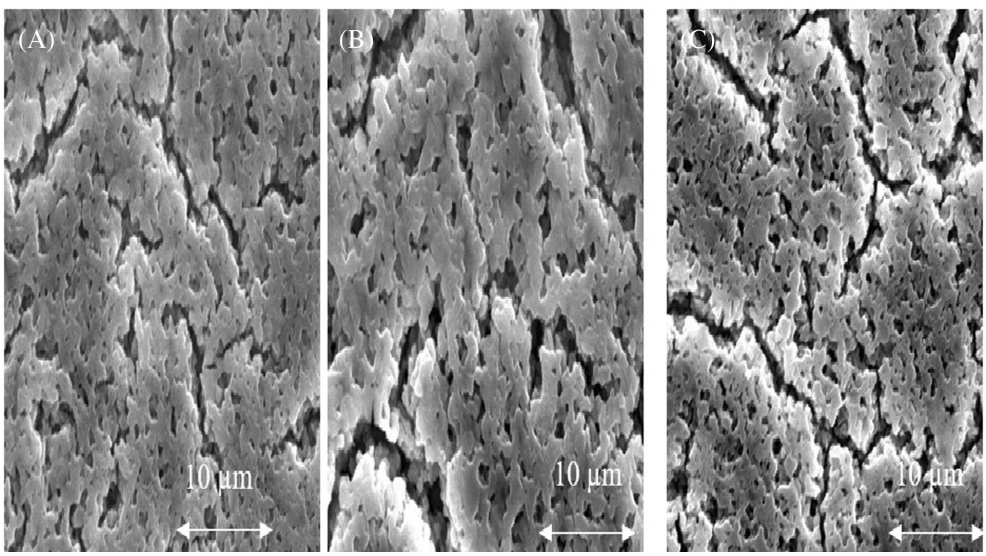

Figure 9. (A) SEM images of PANI-GOx-PPMP/Au electrode. (B) PANI-GOx-PPMP/AuNPs/Au electrode, and (c) PANI-GOx-PPMP-AuNPs/Au electrode.

of 20.0% was observed on day 35. We also determined the performance of the PPMP enzyme on potential changes within 35 days. As shown in Fig. 8(B), a loss of potential of 0.2% was observed on day 35. As a result, it was found that the enzyme was not detached from the electrode surface. Thus, the activity of the enzyme is well protected from the electrode surface.

Morphology studies

The surface morphology of the PANI-GOx-PPMP-AuNPs/Au electrode compared to the PANI-GOx-PPMP/Au electrode and the PANI-GOx-PPMP/AuNPs/Au electrode were investigated through SEM (Fig. 9). The SEM images show a relatively amorphous structure of PANI. The SEM image of the PANI-GOx-PPMP/Au electrode (Fig. 9A) indicates only a polyaniline granular morphology. Figure 9(B) illustrates a cauliflower-like morphology of polyaniline for the PANI-GOx-PPMP/AuNPs/Au electrode. By contrast, the SEM image of the PANI-GOx-PPMP-AuNPs/Au electrode (Fig. 9C) indicates a different morphology when the gold nanoparticles are incorporated into the polyaniline matrix during electropolymerization and formation of the nanocomposite. The image shows the porous, closely packed, and concave membrane, allowing for an easy transfer and better conductivity for the PANI-GOx-PPMP-AuNPs/Au electrode.

CONCLUSIONS

The results of the present study show that the biosensors that contain AuNPs provide a larger surface area, greatly increase the electrocatalytic activity, facilitate high enzyme loading, and lower the LOD. Under optimal conditions, the developed PANI-AuNPs-GOx-PPMP/Au electrode exhibits a linear range from 0.005 to 16.0 mM with a detection limit of 0.001 mM. The demonstrated biosensor is a feasible, reproducible, cost-effective, simple, sensitive, selective, and stable enzyme-based biosensor with a simple immobilization procedure. Additionally, this biosensor allows the determination of glucose in the presence of AA, DA, aspartame, and caffeine at a concentration of up to 0.1 mM. This biosensor can easily compete with any horseradish peroxidase-based biosensor. Future studies will be focused on micro-sized sensitive electrochemical amperometric biosensors for the detection of glucose in real samples.

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CONFLICT OF INTEREST

EEH is the chief scientific officer of Infinite Enzymes, the source of the PPMP. The remaining authors declare that they have no conflicts of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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