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Development of Non-Enzymatic Glucose Sensor Using Ag-Au Bimetallic Modified Pencil Lead Electrode with Voltammetry Method

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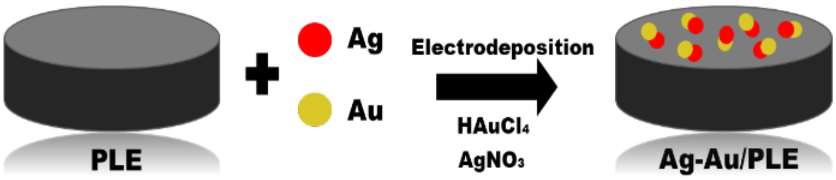
GRAPHICAL ABSTRACT



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ABSTRACT

Glucose is an essential monosaccharide that serves as the primary energy source in the human body. An imbalance in glucose levels can lead to severe metabolic disorders, such as diabetes mellitus. Therefore, effective methods for analyzing and monitoring glucose levels are crucial for early detection and optimal disease management. This study developed a non-enzymatic glucose sensor based on a Pencil Lead Electrode (PLE) modified with silver-gold (Ag-Au) bimetallic using the electrodeposition method via cyclic voltammetry. This modification aims to enhance sensitivity and cost efficiency in glucose detection compared to enzymatic sensors, which are prone to denaturation and higher production costs. Electrodeposition was performed within a potential range of +1.6 V to -0.4 V to achieve an optimal bimetallic coating on the PLE surface. The Ag-Au/PLE electrode exhibited the best electrochemical response for glucose detection, with oxidation and re-oxidation peaks at +0.01 V in 0.1 M KOH as the supporting electrolyte. The optimal condition was achieved with five electrodeposition cycles, yielding a correlation coefficient 0.9984 and a detection limit of 0.206 mM, indicating high sensitivity and accuracy. These results demonstrate that Ag-Au/PLE electrodes have great potential as reliable, sensitive and cost-effective non-enzymatic glucose sensors.

1. INTRODUCTION

Glucose is a monosaccharide (simple sugar) and one of the essential biomolecules that serves as the primary energy source in the human body [1]. Uncontrolled glucose concentrations in the blood can lead to metabolic disorders, including diabetes mellitus [2]. Therefore, developing effective, sensitive, and accurate glucose detection methods is crucial [3, 4]. Various analytical methods have been employed to detect glucose levels, including High-Performance Liquid Chromatography [4], Gas Chromatography [5], Spectrophotometers [6], Calorimetry [8, 9], and electrochemical methods [10, 11]. Electrochemical methods offer a simpler, more sensitive, and cost-effective solution [3]. Unlike other techniques, electrochemical methods do not require complex and expensive instruments, enabling immediate detection without requiring extensive extraction procedures or sample preparation, making them highly suitable for rapid analysis [3].

Most major commercial glucose sensors available today are enzyme-based and utilize glucose oxidase (GOx) to catalyze glucose reactions [11]. Although enzyme-based glucose sensors offer high specificity and fast response, they have certain limitations, such as dependence on enzymes, which are prone to environmental factors like temperature, pH, and the presence of disruptors that can reduce catalytic activity [12, 13]. Additionally, enzyme degradation over time can further decrease the accuracy and reliability of the sensor [14]. To address these limitations, research on non-enzymatic glucose sensors based on electrochemical methods continues to advance [3]. Non-enzymatic glucose sensors offer higher stability and lower production costs than enzyme-based sensors [3]. These sensors utilize metal nanoparticles and carbon nanotubes to interact directly with glucose without requiring an enzymatic reaction. Various types of carbon, metal, and metal oxide nanomaterials have been widely explored for non-enzymatic glucose sensors, including Cu(NP)/PGE [14], MnOxides/PE [15], PANI/PG [16], Ag/CuO NFs/ITO [17], AuNPs/GCE [18], Ag-Au/Sol [19], Ag-Au/SiO_2 [20], Pt-CNTs/TiO_2 [21], Cu-Ag/GCE [22], and others. This study employs a combination of noble metals, specifically Ag and Au, which exhibit catalytic activity, enhancing electron transfer and active sites [4, 19] compared to single-metal systems. The Ag-Au bimetallic modification is applied to the surface of a Pencil Lead Electrode (PLE), a carbon-based working electrode that offers good electrical conductivity, easy availability, low cost, and simple cleaning procedures [23].

In this study, a glucose sensor was developed for the first time using a Pencil Lead Electrode (PLE) modified with an Ag-Au bimetallic coating. The PLE modification with Ag-Au bimetallic was performed using the electrodeposition method on the electrode surface via the cyclic Voltammetry technique. This research presents an efficient, sensitive, rapid, and cost-effective analysis method.

2. EXPERIMENTAL METHODS

2.1. Tools and Materials

This study utilized various chemicals, including D-Glucose anhydrous (Merck), $HAuCl_4.4H_2O$ (Nacalai Tesque Japan), $AgNO_3$ (Merck), KOH (Merck), H_2SO_4 (Merck), $K_3[Fe(CN)_6]$ (Merck), KNO_3 (Merck) and distilled water (Aquadest). The electrochemical measurements were conducted using an e-Daq potentiostat with a three-electrode system consisting of an Ag/AgCl reference electrode, a platinum wire counter electrode, and a Kokuyo Pencil Lead Electrode (PLE) (0.9 mm, 2B) as the working electrode.

2.2. Ag-Au/PLE preparastion by electrodeposition method

Ag, Au, and Ag-Au modification on PLE was performed using the electrodeposition method. The Au/PLE preparation involved immersing the PLE electrode in a solution of 0.4 mM $AuHCl_4.4H_2O$ and 0.01 M H_2SO_4, followed by applying a potential ranging from +1.6 V to 0 V at a scan rate of 100 mV/s for five cycles using the cyclic voltammetric (CV) method. The resulting electrode was designated as a gold-layer-modified PLE. Similarly, the Ag/PLE preparations were carried out by immersing the PLE electrode in a solution of 0.4 mM $AgNO_3$ and 0.01 M H_2SO_4. The potential was adjusted from +1.2 V to 0 V at a scan rate of 100 mV/s for five cycles using the CV method. The electrode was designated as a silver-layer-modified PLE. For Ag-Au/PLE preparation, the PLE electrode was immersed in a solution containing 0.4 mM $AuHCl_4.4H_2O$, 0.2 mM $AgNO_3$, and 0.01 M H_2SO_4. The potential was adjusted from +1.6 V to -0.4 V at a scan rate of 100 mV/s for five cycles using the CV method. The resulting electrode was designated as an Ag-Au bimetallic-modified PLE, as shown in Figure 1.

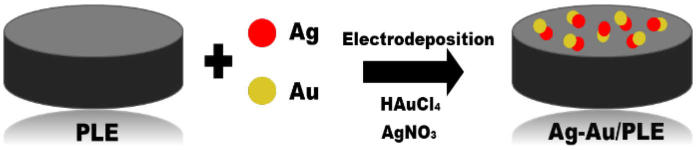

Figure 1. Illustration of Pencil Lead Electrode modification with Ag-Au bimetallic.

2.3. Electrochemical Measurement of Working Electrode Variation on $K_3[Fe(CN)_6]$ solution by Cyclic Voltammetry Method

The Electrochemical measurement of PLE, Ag/PLE, Au/PLE, and Ag-Au/PLE electrodes was performed using cylclic voltammetry in a 2 mM $K_3[Fe(CN)_6]$ solution with 0.1 M KNO_3 as the supporting electrolyte. Measurements were conducted by applying a scan potential ranging from +0.6 V to -0.2 V at a scan rate of 100 mV/s.

2.4. Electrochemical Measurements on Working Electrode Variations on Glucose analytes by Cyclic Voltammetry method

The Electrochemical measurement of PLE, Ag/PLE, Au/PLE, and Ag-Au/PLE electrodes was performed using cyclic voltammetry in a 5 mM glucose analyte solution with 0.1 M KOH as the supporting electrolyte. Measurements were conducted by applying a scan potential ranging from +1 V to -0.6 V at a scan rate of 100 mV/s.

2.5. Effect of Electrodeposition Cycle Number on Glucose Measurement by CV Method

The PLE will be electrodeposited with a bimetallic concentration ratio of Ag-Au (0.2 mM : 0.4 mM). The number of electrodeposition cycles will vary, with variations of 1, 5, and 10 cycles. Measurements will be performed using the CV method with a scan potential ranging from +1.6 V to -0.4 V at a scan rate of 100 mV/s. The modified electrode will then measure a 5 mM glucose analyte using the CV method with a potential scan ranging from +1 V to -0.6 V.

3. RESULTS AND DISCUSSIONS

3.1. Modification of PLE with Bimetallic Ag-Au by Electrodeposition

The modification of the PLE electrode surface with Ag-Au bimetallic was performed by electrodeposition in a solution containing 0.4 mM $HAuCl_4$, 0.2 mM $AgNO_3$, and 0.01 M H_2SO_4 for 5 cycles. The appearance of oxidation and reduction peaks in the voltammogram (figure 2) confirms the successful deposition of the Ag-Au bimetallic on the electrode surface, which is designated as Ag-Au/PLE.

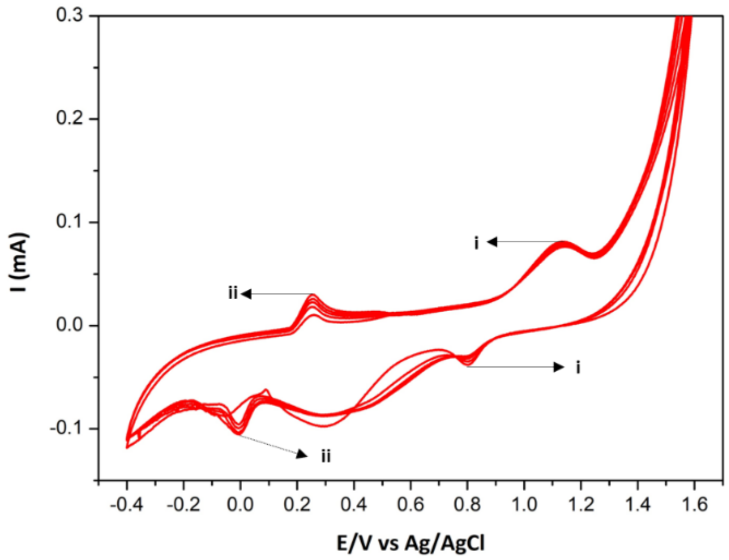

Figure 2. Cyclic voltammogram of Ag-Au/PLE electrodeposition from PLE modified in a solution of 0.4 mM $HAuCl_4$, 0.2 mM $AgNO_3$ and 0.01 M H_2SO_4 at a scan rate of 100 mV/s.

The voltammogram in Figure 2 shows the presence of oxidation peaks and reduction peaks of Ag-Au/PLE with the reaction equation [24, 25]:

Oxidation: i. $Au_{(s)} + 4Cl^{-} \rightarrow AuCl^{4-}_{(aq)} + 3e^{-}$ (1)
ii. $Ag_{(s)} \rightarrow Ag^{+}_{(aq)} + e^{-}$ (2)

Reduction: i. $AuCl^{4-}_{(aq)} + 3e^{-} \rightarrow Au_{(s)} + 4Cl^{-}$ (3)
ii. $Ag^{+}_{(aq)} + e^{-} \rightarrow Ag_{(s)}$ (4)

The voltammogram shows the reduction potential Ag at 0 V with a current of 0.15 mA and the oxidation potential value at 0.25 V with a current of 0.08 mA using Ag/AgCl as the reference electrode. Meanwhile, the Au reduction peak appears at a potential range of +0.65 to +0.8 V with a current of 0.14 mA, and the Au oxidation peak appears at +1.1 V with a current of 0.23 mA. The appearance of oxidation and reduction peaks in the voltammogram confirms the successful deposition of Ag-Au bimetallic on the surface of PLE, designating the electrode as an Ag-Au bimetallic modified pencil lead electrode. The electrodeposition method offers advantages such as simplicity, cost efficiency, and the ability to produce materials with hardness and corrosion resistance properties similar to those of pure metals [26].

3.2. Comparison of Electrode Variation Response to Detection $[Fe(CN)_6]^{3-/4-}$

The electrochemical response of PLE, Ag/PLE, Au/PLE, and Ag-Au/PLE electrodes to 2 mM $[Fe(CN)_6]^{3-/4-}$ in a 0.1 M KNO_3 solution was measured using a potential scan from +0.6 to -0.2 V at a scan rate of 100 mV/s.

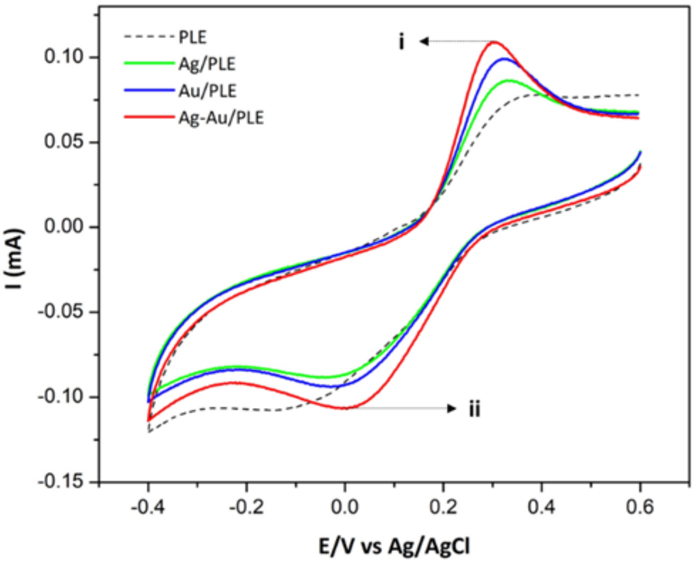

Figure 3. Cyclic voltammogram on working electrode variations of PLE (--), Ag/PLE (green line), Au/PLE (blue line), and Ag-Au/PLE (red line) in 2 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KNO_3 with a scan rate of 100 mV/s.

A comparison of the redox response results of $[Fe(CN)_6]^{-3/-4}$ can be seen on a cyclic voltammogram (Figure 3), with the reaction equation:

Oxidation: $[Fe(CN)_6]^{-4}_{(aq)} \rightarrow [Fe(CN)_6]^{-3}_{(aq)} + e^{-}$ (5)

Reduction: $[Fe(CN)_6]^{-3}_{(aq)} + e^{-} \rightarrow [Fe(CN)_6]^{-4}_{(aq)}$ (6)

The Voltammograms demonstrate the superior performance of Ag-Au/PLE compared to PLE, Ag/PLE, and Au/PLE. This enhancement is attributed to the synergistic effect between Ag and Au, which increases the electrode's active surface area. The high electroactive area is determined based on the Randles-Sevcik equation (7):

$$I_p = 2.69 \text{ x } 10^5 A D^{1/2} n^{3/2} v^{1/2} C \quad (7)$$

Where A represents the electrode area (cm²), v is the scan rate (V/s), and (n) denotes the number of electrons involved in the transfer process. The results of this study confirm the occurrence of heterogeneous electron transfer involving a single electron (n=1). The solution concentration (C) used is 2 mM, while the diffusion coefficient (D) is 6.057 × 10^{-6} cm²/s [27]. The electrode areas obtained for each electrode are 0.048 cm² for bare PLE, 0.055 cm² for Ag/PLE, 0.061 cm² for Au/PLE, and 0.077 cm² for Ag-Au/PLE. This indicates that more active sites are available for interaction with redox species, as evidenced by higher detected currents [27].

3.3. Comparison of Electrode Variation Response to Glucose Detection

A comparison of the responses of PLE, Ag/PLE, Au/PLE, and Ag-Au/PLE electrodes to glucose was conducted to determine which electrode performed better in glucose detection. Measurements were carried out in a 5 mM of glucose solution with 0.1 M KOH using a scan potential of +1 V to -0.6 V. The measurement results are presented in figure 4.

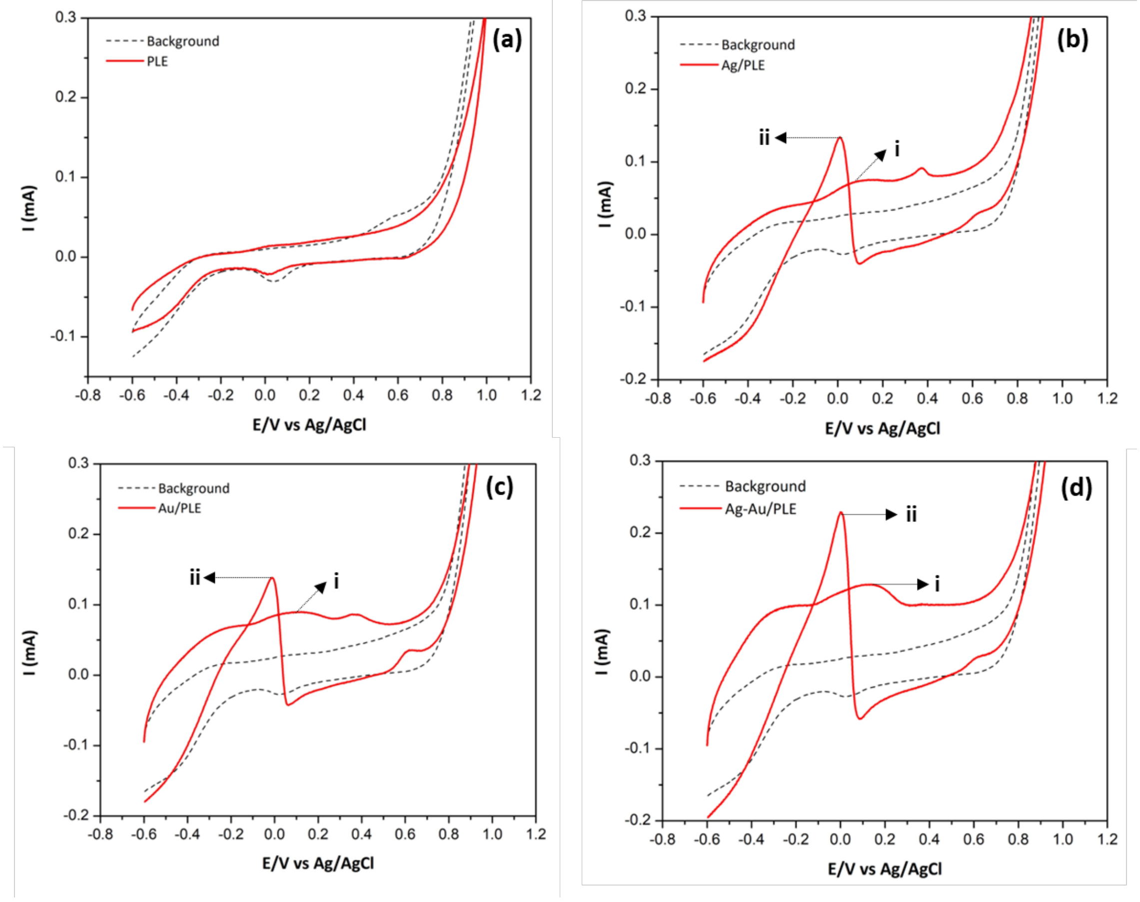

Figure 4. The cyclic voltammogram shows the oxidation(i) and re-oxidation(ii) peaks for different working electrodes: (a)PLE, (b)Ag/PLE, (c)Au/PLE, and (d)Ag-Au/PLE in 5 mM Glucose and 0.1 M KOH at a scan rate of 100 mV/s.

Based on the voltammogram in Figure 4, the electrochemical activity of different electrodes varies in glucose detection. PLE does not exhibit redox behavior toward glucose detection, unlike the modified electrodes. In contrast, Ag/PLE and Au/PLE generate a more defined current response to glucose oxidation and re-oxidation within the potential range of +0.1 V to -0.1 V. However, the Ag-Au/PLE electrode produces the highest oxidation and re-oxidation peak current, where glucose undergoes deprotonation and oxidizes to gluconolactone, releasing electrons ($2e^-$) and hydroxide ions (OH^-) according to the reaction (Glucose + $2OH^-$ → Gluconolactone + H_2O + $2e^-$) [20]. The released electrons are transferred to the electrodes, generating a current of 0.2285 mA. The high oxidation and re-oxidation peak current indicates the electrocatalytic effect of the Ag-Au bimetallic, which enhances the current response to glucose electro-oxidation compared to electrodes containing only Ag or Au [19]. The improved electrochemical performance of Ag-Au/PLE is attributed to the synergistic effect between Ag and Au, which are well-deposited on the electrode surface. Ag has a high affinity for glucose and facilitates electron transfer, while Au accelerates the electron transfer rate during the electrochemical process. Additionally, modifying the electrode with the Ag-Au bimetallic increases its active surface area, enabling more glucose molecules to react, resulting in higher oxidation and re-oxidation currents [18, 27].

3.4. Effect of Variation in Electrodeposition Cycle Number in Glucose Detection

The electrodeposition cycle determines the thickness and distribution of the predeposited Ag-Au bimetal on the electrode surface, directly influencing the electrocatalytic activity in glucose oxidation [28, 29]. Measurements were conducted in a 5 mM glucose solution and 0.1 M KOH with a scan potential ranging from +1 V to -0.6 V. The number of electrodeposition cycles applied varied between 1, 5, and 10 cycles, and the measurement results are shown in Figure 5.

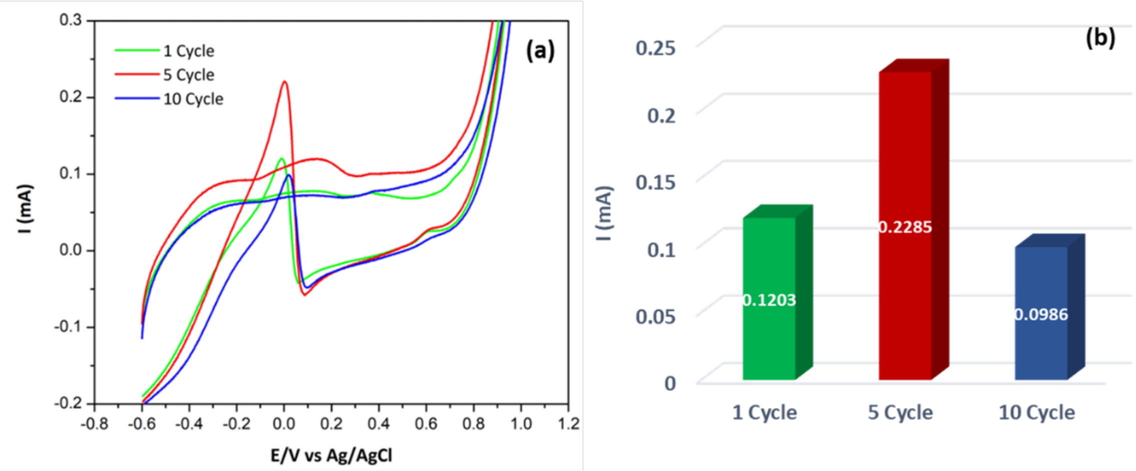

Figure 5. (a) Cyclic Voltammogram of Ag-Au/PLE with varying electrodeposition cycles, (b) Graph of Ag-Au/PLE showing the effect of electrodeposition cycle variations on the measurement of 5 mM Glucose in 0.1 M KOH at a scan rate of 100 mV/s.

The optimal number of electrodeposition cycles is crucial for enhancing the performance of Ag-Au/PLE electrodes in glucose detection. Based on the voltammogram in Figure 5(a) and the graph in Figure 5(b), the optimal condition is achieved at 5 cycles, as indicated by the highest oxidation current of 0.2285 mA. This finding aligns with the study conducted by Hezard et al. (2012), which stated that within the range of 1 to 4 cycles (Optimal at 4 cycles), the formation of tiny, spherical-shaped nanoparticles (NPs) is more favorable, as the electrode exhibits a balance between active surface area, appropriate layer thickness, and uniform distribution [30]. At optimal cycles, the electrode maintains a balance between these factors while ensuring a well-distributed Ag-Au bimetallic layer. This combination results in a higher glucose oxidation current and enhances electrode sensitivity compared to an insufficient or excessive number of cycles. In comparison, at 1 cycle, the measured oxidation current is only 0.1203 mA, while at 10 cycles, a significant decrease is observed, with an oxidation current of 0.0986 mA. An insufficient number of cycles (1 cycle) produces a thin and uneven Ag-Au bimetallic layer, limiting the number of available electrocatalytic active sites and leading to a lower glucose oxidation current [30]. Conversely, an excessive number of cycles (10 cycles) results in an overly thick Ag-Au bimetallic layer, promoting metal particles' agglomeration. Excessive metal accumulation hinders electron transfer by reducing accessibility to active sites responsible for glucose oxidation [30]. Consequently, the electrode's electrochemical response declines, leading to a lower measured oxidation current [28, 29].

The modification of electrodes using the electrodeposition method must be conducted under optimal conditions, particularly in determining the number of electrodeposition cycles, as this parameter plays a crucial role in enhancing electrode stability and efficiency [29, 30]. An optimal number of cycles facilitates the formation of a homogeneous bimetallic layer with a uniform metal distribution, thereby reducing charge transfer resistance and improving the electrode's catalytic activity [29]. This improvement not only enhances the electrode's sensitivity in glucose detection but also strengthens its long-term stability [29]. Therefore, selecting the appropriate number of electrodeposition cycles is critical in developing reliable electrodes for glucose sensor applications.

The calibration curve was obtained using the Linear Sweep Voltammetry (LSV) method with glucose concentration ranging from 0 mM to 5 mM in the 0.1 M KOH as the supporting electrolyte solution. Measurements were conducted over a scan potential range from +1 V to -0.6 V, corresponding to the re-oxidation potential of glucose, with a scan rate of 100 mV/s.

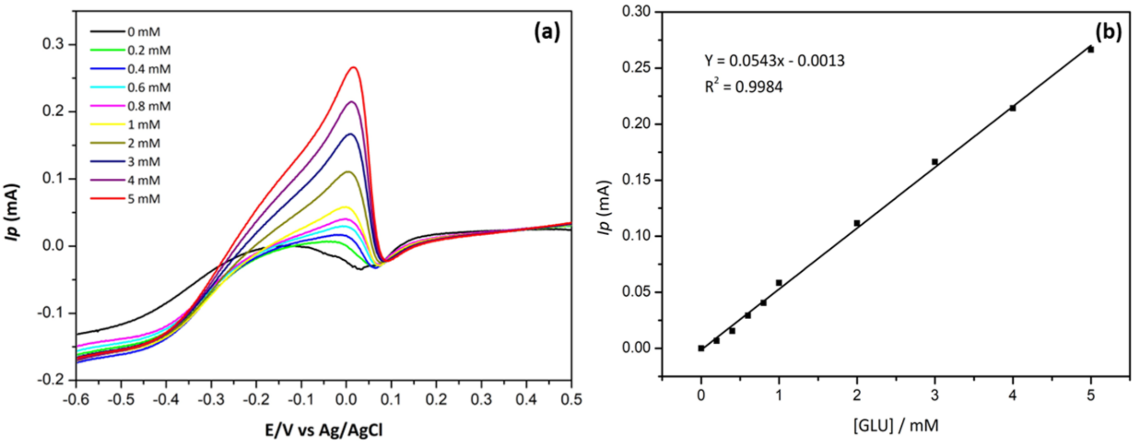

Figure 6. (a) LSV Voltammogram on Ag-Au/PLE in KOH 0.1 M, scan potential +1 V to -0.6 V (b) Glucose Calibration Curve with concentration variation 0 to 5 mM.

Figure 6 presents the voltammogram and linearity of glucose measurement at varying concentrations. The results indicate a strong correlation between glucose concentration and re-oxidation current, demonstrating that higher glucose concentration leads to more significant glucose accumulation on the electrode surface [16, 18]. This trend is also observed in Figure 6, where an increase in glucose concentration results in a higher re-oxidation current. The linear regression equation y = 0.0543x – 0.0013 was obtained based on data analysis, with a correlation coefficient (R^2) of 0.9984, indicating a high degree of linearity. Additionally, the limit of detection (LOD) was determined to be 0.206 mM. These results confirm that Ag-Au/PLE electrodes exhibit good sensitivity and linearity in glucose detection, making them promising candidates for development and application in reliable glucose sensor systems.

4. CONCLUSIONS

Non-enzymatic glucose sensors based on Pencil Lead Electrodes (PLE) modified with Ag-Au bimetals demonstrate optimal performance in glucose detection. The Ag-Au bimetallic electrodeposition process, carried out using the Cyclic Voltammetry method at a potential range of +1.6 V to -0.4 V with 5 cycles, has been proven to produce sensors with high sensitivity and accuracy. This is evidenced by the oxidation peak observed at +0.01 V in a 0.1 M KOH solution, a regression value of 0.9984, and a detection limit of 0.206 mM. Therefore, Ag-Au/PLE electrodes hold significant potential for use as effective and reliable glucose sensors in glucose level analysis and monitoring applications.

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