

Article

# Comparison Study on Electrochemical Determination of Hydrogen Peroxide by GCE Modified With Gold, Palladium and Palladium-Gold Nano Particles

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**Abstract:** Using electrochemical modified electrodes have been proved to be an inexpensive and sensitive method for analysis of wide range of analytes. In this study, a sensitive sensor for the determination of hydrogen peroxide was fabricated based on glassy carbon electrode modified with Pd-Au co-deposit nanoparticles. The modified electrode showed enhanced response to hydrogen peroxide as compared to Pd nanoparticles, Au nanoparticles modified and bare glassy carbon electrodes. Among eleven mixed Pd:Au mole ratio studied (0:1), (0.1:0.9), (0.2:0.8), (0.3:0.7), (0.4:0.6), (0.5:0.5), (0.6:0.4), (0.7:0.3), (0.8:0.2), (0.9:0.1) (1:0); the 0.8:0.2 showed the highest response for signal for electro reduction of H<sub>2</sub>O<sub>2</sub>. The optimization of the experimental conditions such as deposition potential and deposition time, pH for electroanalysis of H<sub>2</sub>O<sub>2</sub> and reduction potential for H<sub>2</sub>O<sub>2</sub> at modified electrodes for amperometric determination was studied. The selected co-deposit demonstrated a large dynamic concentration range ( $5 - 170 \times 10^{-6}$  mol/L) with a limit of detection of  $3.8 \times 10^{-7}$  mol/L under the optimized conditions. The fabricated nanoparticles were applied for analysis of H<sub>2</sub>O<sub>2</sub> in mouth wash. The selected Pd-Au co-deposit was able to detect low concentration of hydrogen peroxide that was not possible to detect by Pd nanoparticles and Au nanoparticles modified glassy carbon electrode.

**Keywords:** Pd-Au Bimetallic Nanoparticles, Pd Nanoparticles, Au - Nanoparticles, Hydrogen

## 1. Introduction

Now a day many types of monometallic and bimetallic NPs of different sizes and compositions are available for electrode surface modification, which facilitate their application in electroanalysis, bringing important advantages<sup>1,2</sup>. The size and morphology of the electrode and the fabrication method used can be very influential on the voltammetric response of the system<sup>3</sup>.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a chemically modified electrode (CME) is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. While CMEs can operate both amperometrically (and voltammetrically) and potentiometrically, they are generally used amperometrically, a faradaic (charge transfer) reaction being the basis of experimental measurement or study<sup>3,4</sup>. Whereas enzyme based electrodes detect the product(s) of a reaction between an immobilized enzyme layer and a reaction substrate in many ways, including both amperometric and potentiometric means.

The study of nanoscale materials in recent years has been extensively studied, particularly with respect to metallic NPs. Underpinning the significance of nano particulate materials in electro-analysis are the very specific properties which may be exhibited at the nanoscale but which are not typical of the corresponding bulk material. These include enhanced diffusion based on convergent rather than linear diffusion at the smaller NPs, high active surface area, improved selectivity, catalytic activity, higher signal-to-noise ratio and unique optical properties<sup>2</sup>. The use of NPs also provides control over the local microenvironment. This can be highly advantageous when incorporating sensitive or biological material into a system. These unique properties make nanomaterials ideally suited for electroanalytical applications. Enhanced convergent mass transport to nano-electrodes facilitates the study of faster electrochemical processes<sup>2</sup>.

At the nanoscale, crystal planes can be exposed which are not accessible at the macro scale, in turn giving rise to improved current responses and catalysis<sup>5</sup>. A few commonly used metals for NPs are gold<sup>6</sup>, silver<sup>1</sup> and platinum<sup>7</sup>. The electro analytical application of such nanomaterials has been found to be quite extensive. This suggests the potential for study of a wide variety of metal NPs, not only typically used electrode materials such as those mentioned above, but also metals such as palladium<sup>7</sup>, ruthenium<sup>8</sup>, nickel, and copper<sup>6</sup>. Furthermore this study was investigated for fabrication of more electro active sensor of H<sub>2</sub>O<sub>2</sub> depending on previous studies modified with different chemicals, such as

myoglobin, hemoglobin, metal complexes, horseradish peroxidases (HRP), and metal hexacyanoferrates have been used for determination of  $\text{H}_2\text{O}_2$ <sup>7-11</sup>, the current study deals with comparison of monometallic NPs of Au and Pd with Pd-Au co-deposit NPs with 5 seconds deposition time applied for electrochemical reduction of  $\text{H}_2\text{O}_2$ . And as we know the amount of metal nanoparticles depends on the length of deposition time, the longer the deposition time more bulk the modified electrode which is thin film and the shorter the time the size of metal nanoparticles small and more active centers present on the electrode surface which is highly sensitive and selective for the given analyte.

## 2. Materials and Methods

### 2.1. Chemicals

Palladium dichloride ( $\text{PdCl}_2$ , 99.9%, Aldrich), Potassium tetrachloroaurate (III) ( $\text{KAuCl}_4$ , 98%, Aldrich), Sodium perchlorate ( $\text{NaClO}_4$ , 98%, Aldrich), Potassium hydroxide (KOH, BDH), Citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ , 99.5%, Wardel chemicals ltd), Sodium chloride ( $\text{NaCl}$ , 99.8% Riedel-de Haen), Hydrochloric acid (HCl, 37%, Riedel-de Haen), Potassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ , 98 %, FINKEM), Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ , 99%, NICE), Potassium iodide (KI, 99%, Riedel-de Haen), Sulfuric acid ( $\text{H}_2\text{SO}_4$ , 98%, Riedel-de Haen), Sodium thio sulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ , Riedel-de Haen), Potassium chloride (KCl, Riedel-de Haen), Hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 30%, Aldrich), Phosphoric acid ( $\text{H}_3\text{PO}_4$ , 98.5 %, FINKEM). Distilled water was used to prepare all aqueous electrolyte solutions throughout the study.

### 2.2. Instruments

A three-electrode assembled cell was employed, consisting of the modified GCE (3.0 mm diameter) as the working electrode, a platinum-wire as a counter electrode, and Ag/AgCl electrode as a reference electrode. The polishing kit was used for polishing the working electrode. Electrochemical techniques, including cyclic voltammetry (CV) and Chronoamperometry (CA), were performed using Epsilon EC-Ver 1.40.67 voltammetric analyzer (Bioanalytical Systems, USA). For amperometric measurements magnetic stirrer was used. All experiments were conducted at room temperature.

### 2.3. Palladium Deposition Bath Preparation

A Pd deposition bath consisting of 0.0075 mol/L  $\text{PdCl}_2$  in citrate buffer prepared by dissolving 66.50 mg of  $\text{PdCl}_2$  in 50 mL of pH 2 citrate buffer (consisting of 0.03 mol/L citric acid, 0.0082 mol/L HCl, 0.061 mol/L NaCl). The solution was further homogenized by shaking and left overnight in the dark after which period a clear solution was obtained. Finally, citrate buffer solution pH was adjusted to 3 by addition of diluted KOH (0.1 mol/L)<sup>12</sup>.

### 2.4. Gold Deposition Bath Preparation

Gold deposition bath containing a 0.05 mol/L  $\text{KAuCl}_4$  which dissolved in 0.5 mol/L  $\text{H}_2\text{SO}_4$  solution. To prepare the deposition bath, 0.028 mg of  $\text{KAuCl}_4$  was added to 1.5 mL of 0.5 mol/L  $\text{H}_2\text{SO}_4$  solutions. For deposition bath 0.001 mol/L  $\text{KAuCl}_4$  was prepared by diluting 0.1 mL of 0.05 mol/L  $\text{KAuCl}_4$  stock solution in 5 mL 0.5 mol/L  $\text{H}_2\text{SO}_4$ <sup>13</sup>. The reason why Au deposition bath was not prepared by using citrate buffer is due to the reducing ability of citrate buffer for gold.

### 2.5. Pd-Au Co-Deposition Bath Preparation

A 0.05 M  $\text{PdCl}_2$  of palladium and 0.05 mol/L  $\text{KAuCl}_4$  of gold stock solution was prepared by dissolving both in 0.5 mol/L  $\text{HNO}_3$  solution separately. To prepare 0.05 mol/L  $\text{KAuCl}_4$  stock solution of gold 0.028 mg of  $\text{KAuCl}_4$  was dissolved in 1.5 mL of 0.5 mol/L  $\text{HNO}_3$  solution and to prepare 0.05 mol/L  $\text{PdCl}_2$  stock solution of palladium 0.0133 mg of  $\text{PdCl}_2$  was dissolved in 1.5 mL of 0.5 mol/L  $\text{HNO}_3$  solution. Then for deposition bath 0.001 mol/L  $\text{KAuCl}_4$  was prepared by diluting 0.1 ml of 0.05 mol/L  $\text{KAuCl}_4$  stock solution in 5 ml 0.5 mol/L  $\text{HNO}_3$  and the same procedure was used for preparation of palladium deposition bath with 0.001 mol/L  $\text{PdCl}_2$ . After preparing 0.001 mol/L  $\text{PdCl}_2$  and 0.001 mol/L  $\text{KAuCl}_4$  solution from their salts by dissolving in 0.5 mol/L  $\text{HNO}_3$  solution, different mole ratios of Pd-Au co-deposition deposition bath prepared by taking an appropriate volume for each ratios.

### 2.6. Electrode Preparation

#### 2.6.1. Electrode conditioning

A glassy carbon rod 3 mm in diameter (GCE) was mechanically polished with of polishing suspensions on BAS polishing cloth. The electrode was further cleaned electrochemically. GCE was conditioned by potential scanning from -1.1 V to 1.2 V in 0.1 mol/L  $\text{NaClO}_4$  for at least five complete scans at  $0.5 \text{ Vs}^{-1}$ . The prepared electrodes were used immediately after mechanical polishing and electrochemical cleaning.

#### 2.6.2. Metal deposition

Palladium, Gold and Palladium-Gold electrode was prepared by potentiostatic deposition of  $\text{PdCl}_2$ ,  $\text{KAuCl}_4$  and  $\text{PdCl}_2$  -  $\text{KAuCl}_4$  mixture on cleaned GCE from Pd, Au and Pd-Au deposition bath respectively.

#### 2.6.3. Au-NP modified GC electrode fabrication

On a cleaned and electrochemically conditioned GCE a solution of  $\text{KAuCl}_4$  was reduced by stepping the potential from 1.10 to 0 V over 5 s. Thus, the Au nanoparticles modified GCE has been fabricated. Then the Au-NPs modified GCE was rinsed with distilled water and applied for the further electrochemical studies.

#### 2.6.4. Pd-NP modified GC electrode fabrication

On a cleaned and electrochemically conditioned GCE a solution of PdCl<sub>2</sub> was reduced by stepping the potential from 1.10 to 0 V over 5 s. Thus, the Pd nanoparticles modified GCE has been fabricated. Then the Pd-NPs modified GCE was rinsed with distilled water and applied for the further electrochemical studies.

#### 2.6.5. Pd-Au modified GC electrode fabrication

From solutions of 0.001 mol/L PdCl<sub>2</sub> and KAuCl<sub>4</sub> eleven mixtures of Pd:Au with mole ratios of (0:1), (0.1:0.9), (0.2:0.8), (0.3:0.7), (0.4:0.6), (0.5:0.5), (0.6:0.4), (0.7:0.3), (0.8:0.2), (0.9:0.1), (1:0) were prepared. On a cleaned and electrochemically conditioned GCE a solution each mixtures was reduced by stepping the potential from 1.10 to 0 V over 5 s.

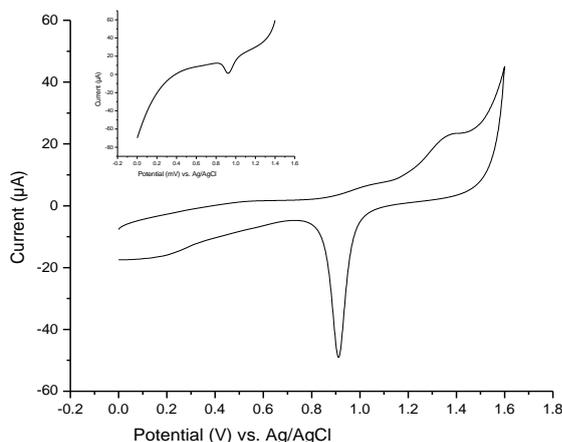
Thus, the Pd-Au nanoparticles modified GCE has been fabricated. Then the Pd-Au NPs modified GCE was rinsed with distilled water and applied for the further electrochemical studies.

### 3. Results and Discussion

#### 3.1. Characterization of Au Cyclic Voltammogram

A primarily study of electrochemical Au deposition was performed by cyclic voltammetry in unstirred solution. Cyclic voltammogram (CV) of Au is important for obtaining oxidation and reduction potentials for Au deposition process. The voltammogram shows some basic information, the characteristic features of Au reduction and oxidation.

Figure 1 shows the cyclic voltammogram (CV) of Au deposition process. The voltammogram shows the reduction of Au<sup>3+</sup> takes place at around 0.921 V and oxidation of reduced Au atom is around 1.4 V. The inset linear sweep voltammetry shows the gold deposition on GCE surface that is the reduction of gold around 0.921 V in 0.5 mol/L H<sub>2</sub>SO<sub>4</sub>.



**Figure 1:** Cyclic voltammogram of Au deposition on a GC surface from a KAuCl<sub>4</sub> in 0.5 mol/L H<sub>2</sub>SO<sub>4</sub> solution at scan rate 0.5 V s<sup>-1</sup>.

### 3.2. Optimization of Experimental Parameters for Au-NP Modified GCE Effect of pH

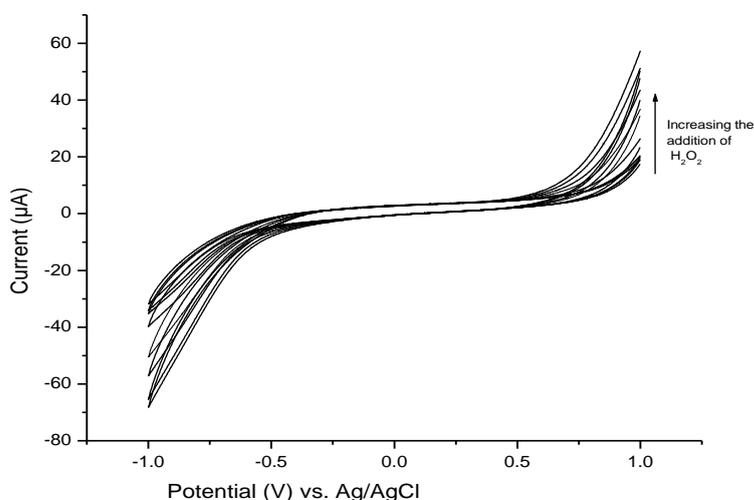
In order to improve the performance of the modified electrode, the pH may influence the response of the electrode. Therefore, the effect of pH was studied. The effect of the pH of supporting electrolyte on the electrode response was tested in the pH range of 5.8 to 8.0. Therefore among all pH 7.6 showed good response and it was selected for the experiments.

### 3.3. Influence of Deposition Time

The amount of metal particles on the surface of the electrode has great effect on the performance of the modified electrode. Therefore, the amount of metal particles on the electrode surface depends on the deposition time. So that, the deposition time should be optimized. With increasing of Au-NP deposition time for modified GCE the CV response increases up to 5 seconds. The further increase of deposition time led to decrease of the response. Therefore, Au-NPs are deposited on the GCE for 5 seconds and electrodes prepared under this condition are referred as Au-NPS modified GC.

### 3.4. Electrochemical Behavior of Hydrogen Peroxide at Bare GCE

Figure 2 shows the electrochemical response of glassy carbon electrode to additions of hydrogen peroxide in phosphate buffer, with scan rate of  $0.5 \text{ Vs}^{-1}$ . The electrochemical responses were run over a range of scan rates but no significant voltammetric currents were observed. No obvious peak corresponding to the reduction and oxidation of hydrogen peroxide is observed at bare GCE. This tells us the bare GCE is not able to detect  $\text{H}_2\text{O}_2$  and it is inert to  $\text{H}_2\text{O}_2$ .

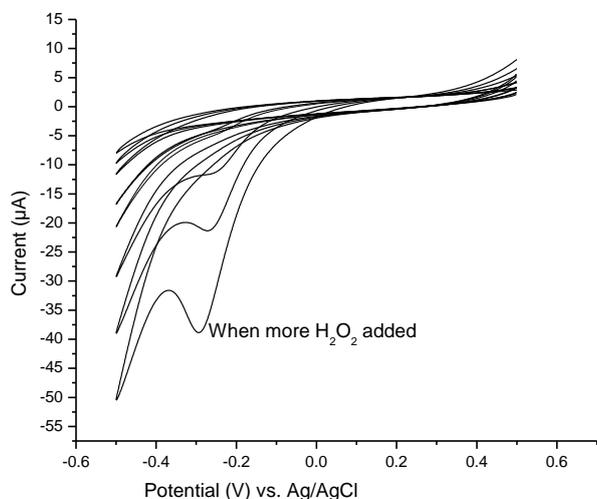


**Figure 2:** Cyclic voltammogram detailing the response of a GCE to additions of  $\text{H}_2\text{O}_2$  (0, 0.1, 0.5, 1, 5, 10, 15, 20 and  $25 \times 10^{-3} \text{ mol/L}$ ). Run at a scan rate of  $0.5 \text{ Vs}^{-1}$  in PBS (0.1 mol/L).

### 3.5. Electrochemical Behavior of $\text{H}_2\text{O}_2$ at Au-NPs Modified on GCE

Figure 3 show the response of the GCE modified by Au-NPs to additions of hydrogen peroxide

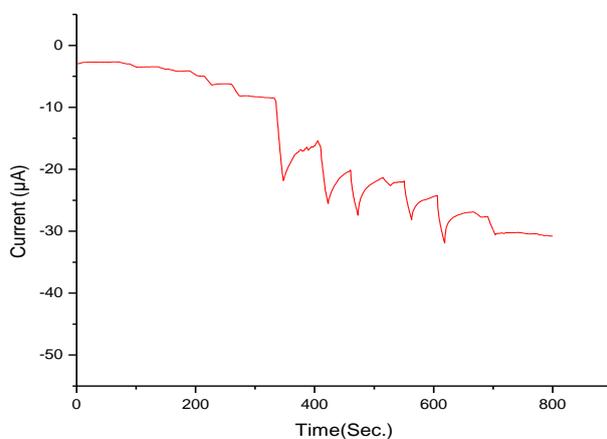
(H<sub>2</sub>O<sub>2</sub>). It can be concluded that the presence of the deposited Au-NPs on the surface of the GCE will not allow the determination of hydrogen peroxide at lower concentrations. However, at high concentrations of H<sub>2</sub>O<sub>2</sub> Au modified GCE gave a current response for reduction of H<sub>2</sub>O<sub>2</sub> but higher current response was not observed.



**Figure 3:** Cyclic voltammogram detailing the response of Au-NPs to additions of H<sub>2</sub>O<sub>2</sub> (0, 0.1, 0.5, 1, 5, 10, 15, 20 and 25 × 10<sup>-3</sup> mol/L). Run at a scan rate of 0.5 V s<sup>-1</sup> in PBS (0.1 mol/L).

### 3.6. Amperometric Determination of H<sub>2</sub>O<sub>2</sub> at Au-NPs

As shown in the figure 4 it was observed that during successive additions of H<sub>2</sub>O<sub>2</sub> the current-time response of the Au-NPs modified GCE increases. But the amperometric response of the analyte for Au-NPs modified GCE is lower. This is due to less catalytic activity of Au-NPs for the reduction of H<sub>2</sub>O<sub>2</sub><sup>14</sup>.



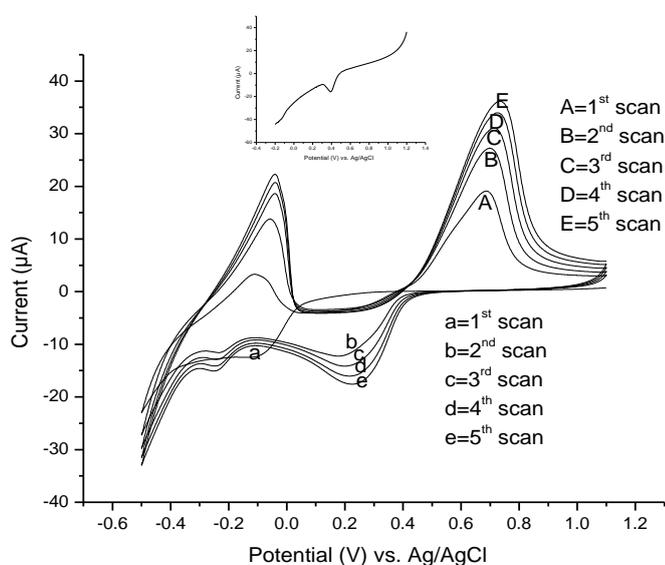
**Figure 4:** A current-time response curve stirring with successive additions of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0... and 130.0 × 10<sup>-6</sup> mol/L H<sub>2</sub>O<sub>2</sub> for glassy carbon electrode modified with Au-NPs in 10.0 mL 0.1 mol/L pH 7.6 PBS.

### 3.7. Characterization of GCE Modified by Pd on Voltammogram

A primarily study of electrochemical Pd deposition was performed by cyclic voltammetry in unstirred solution. Cyclic voltammogram (CV) of Pd is important for obtaining oxidation and reduction potentials for Pd deposition process. The voltammogram shows some basic information, the characteristic features of Pd reduction and oxidation.

Therefore figure 5 shows the cyclic voltammogram of Pd metal surface deposition process at GCE. Here the CV shows the characteristic current features of Pd reduction (around 0.4 V), Pd oxide formation (around 0.73 V) figure 5. The inset linear sweep voltammetry in 1 mol/L H<sub>2</sub>SO<sub>4</sub> showed palladium oxide formation followed by reduction for the deposited palladium particles on a glassy carbon electrode and this confirms the deposition of palladium on glassy carbon electrode.

On scanning the potential in negative direction, Pd particles are deposited on the electrode surface and the peak around -0.2 V shows the reduction process of protons to hydrogen, which are adsorbed on the Pd surface. This reduction peak shows the hydrogen adsorption process on the Pd surface. During the positive potential scanning process, the peak around -0.1 V is appears due to the oxidation of hydrogen atoms. In the next step the deposited Pd particles are further oxidized to Pd<sup>2+</sup> to form a Pd oxide layer (0.73 V) on the electrode surface. The formed Pd oxides are further reduced on the negative-going scans, leading back to Pd particles with hydrogen adsorption process. During this repetitive cycling process, all the peaks are found growing which confirms the Pd modified GCE has been fabricated.



**Figure 5:** Repeated cyclic voltammogram of Pd electrodeposited on GCE from citrate buffer solution pH 3 containing PdCl<sub>2</sub> and potential scan between 1.1 and -0.6 V for five cycles scan rate of 0.5 Vs<sup>-1</sup>.

### 3.8. Optimization of pH for Pd-NP Modified GCE

In order to improve the performance of the modified electrode, the pH may influence the response of the electrode. The effect of the pH of supporting electrolyte on the electrode response was tested in the range of 5.8 to 8.0. The maximum response was seen at pH 7.4 and this pH was easily selected for the remainder of the experiments.

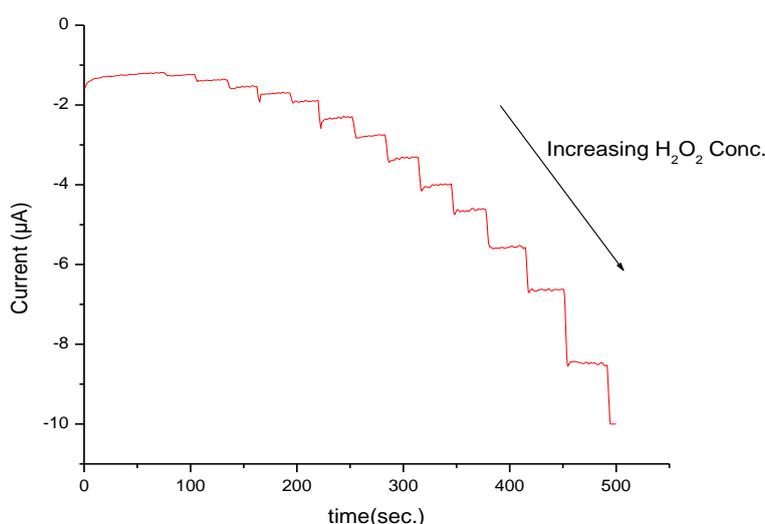
### 3.9. Pd-NPs Optimization of Deposition Time

The amount of metal particles on the surface of the electrode has great effect on the performance of the modified electrode. Therefore, the amount of metal particles on the electrode surface depends on the deposition time. So that, the deposition time should be optimized. The increasing of Pd-NPs deposition time for modified GCE the CV response increases up to 5 seconds. The further increase of deposition time led to decrease of the response. Therefore, Pd-NPs are deposited on the GCE for 5 seconds and electrodes prepared under this condition are referred as Pd-NPs modified GC.

### 3.10. Electrochemical Behavior of $H_2O_2$ at Pd-NPs Modified on GCE

#### 3.10.1. Amperometric response of $H_2O_2$ at Pd-NPs modified GCE

Since amperometry under stirred condition is much more sensitive than cyclic voltammetry, this method was employed for determination of  $H_2O_2$  even at much lower concentrations citation needed. Figure 6 displays the current-time response of the Pd-NPs modified GCE with successive injection of hydrogen peroxide at an applied potential of -0.250 V versus reference electrode (Ag/AgCl).

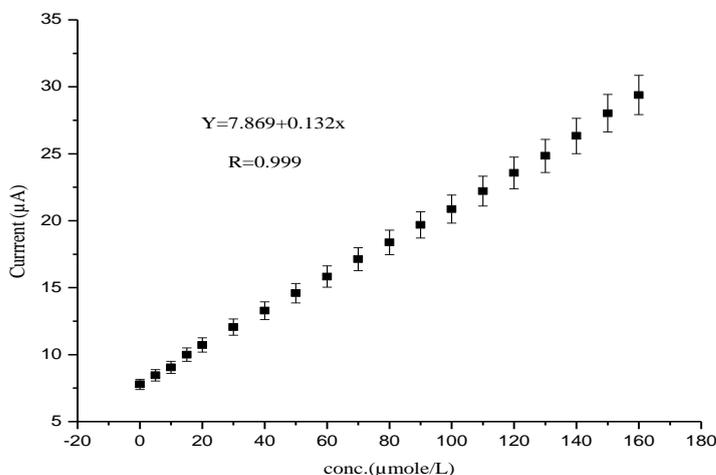


**Figure 6:** A current-time response curve for successive additions of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0, 130.0, ... and  $200 \times 10^{-6}$  mol/L  $H_2O_2$  for glassy carbon electrode modified with Pd-NPs in 10.0 mL 0.1 mol/L pH 7.4 PBS stirring with 550 revolution per second.

As it is shown in the figures 6 a well-defined response was observed during the successive additions of  $\text{H}_2\text{O}_2$ . The current-time response of the Pd-NPs modified GCE for successive additions of  $\text{H}_2\text{O}_2$  increases stepwise with each additions of hydrogen peroxide.

The calibration curve shows that the response of Pd-NPs modified GCE to  $\text{H}_2\text{O}_2$  has a linear relationship in the range of  $5 \times 10^{-6}$  -  $200 \times 10^{-6}$  mol/ L with the regression equation:

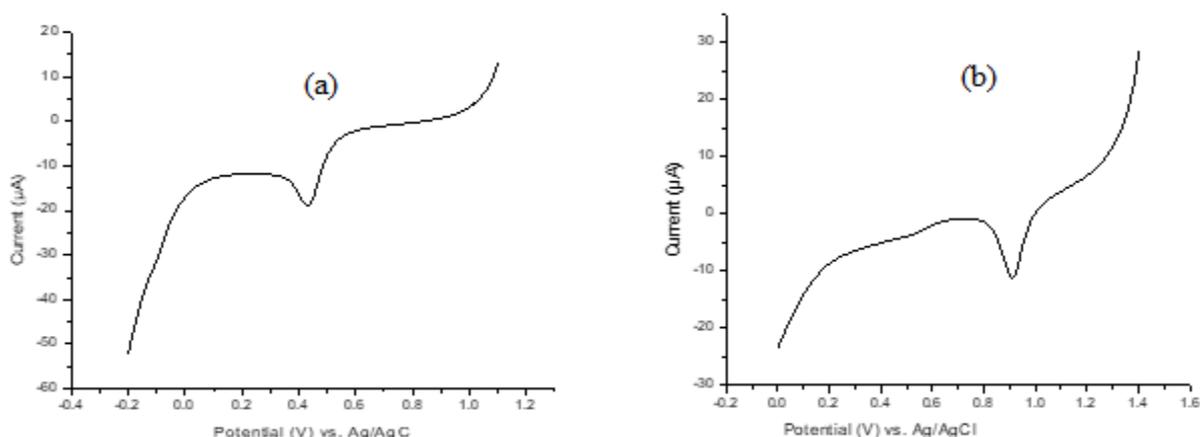
$$y = 0.132x + 7.869, R = 0.999$$



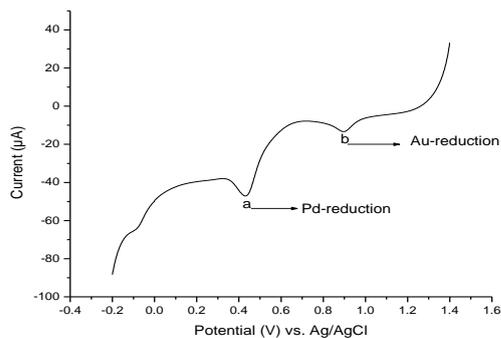
**Figure 7:** Calibration curve for Pd-NPs modified GCE for determination of  $\text{H}_2\text{O}_2$ .

### 3.11. Characterization of Pd-Au Co-deposit modified GCE

Figure 8 (a) shows the linear sweep voltammogram (LSV) of Pd nanoparticles deposition with potential window 1.1 V is applied for 5 s and scanned in a negative direction that is -0.2 V and the characteristic current features of Pd reduction around (0.4V), and (b) shows the linear sweep voltammogram (LSV) of gold nanoparticles deposition with potential window 1.4 V is applied for 5 s and scanned in a negative direction that is -0.2 V.



**Figure 8:** Linear sweep voltammetry of Pd-Au (0.8:0.2) oxide formation followed by reduction for the deposited (0.8:0.2) Pd-Au particles on a GCE surface. A constant potential of 1.1 V is applied for 5 s and scanned in a negative direction at  $0.5 \text{ Vs}^{-1}$  in 1 mol/L  $\text{H}_2\text{SO}_4$ . a) deposited Pd from 0.001 mol/L  $\text{PdCl}_2$  in 0.5 mol/L  $\text{HNO}_3$ , and b) deposited Au 0.001 mol/L  $\text{KAuCl}_4$  in 0.5 mol/L  $\text{HNO}_3$  solution.

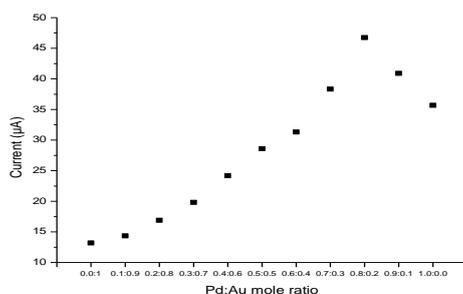


**Figure 9:** LSV of Pd-Au (0.8:0.2) co-deposit oxide formation followed by reduction for the deposited Pd-Au (0.8:0.2) co-deposit NPs on a GCE. A constant potential of 1.1 V is applied for 5 s and scanned in a negative direction at  $0.5 \text{ Vs}^{-1}$  in 1 mol/L  $\text{H}_2\text{SO}_4$ . a) from 0.001 mol/L  $\text{PdCl}_2$  in 0.5 mol/L  $\text{HNO}_3$ , and b) deposited Au 0.001 mol/L  $\text{KAuCl}_4$  in 0.5 mol/L  $\text{HNO}_3$  solution.

For Pd-Au co-deposit both Pd and Au metals should be present on the electrode surface. The presence of palladium and gold deposits is confirmed by the formation of a palladium and gold oxide layer followed by its reduction. The peak potential for reduction of palladium oxide is +0.40 V and gold oxide is +0.921 V.

### 3.12. Pd-Au ratio comparison

First electrochemical deposition of metal nanoparticles, and then optimization and characterization of the GCE modified with different atomic ratio of Pd-Au NPs co-deposit was carried out by using cyclic voltammetry by reading the reduction current response for hydrogen peroxide. That is to which one is the most electro active for the analyte by observing the highest reduction current response for the analyte. Therefore among these 11 modifiers the maximum reduction current was obtained for Pd-Au (0.8:0.2) modifier.



**Figure 10:** Plot to the response of Pd-Au 1, (0:1) ,2,( 0.1:0.9),3, (0.2:0.8 )4,( 0.3:0.7), 5,(0.4:0.6),6,(0.5:0.5), 7,(0.6:0.4), 8, (0.7:0.3), 9,( 0.8:0.2),10, (0.9:0.1), and 11 (1:0) NPs co-deposit modified GCE to pH 7.6 values containing 0.02 mol/L  $\text{H}_2\text{O}_2$  respectively. Run at a scan rate of  $0.5 \text{ Vs}^{-1}$ .

As reported<sup>14</sup>, hydrogen peroxide is consumed simultaneously by two alternative reaction pathways, namely, (i) heterogeneous catalytic decomposition and (ii) electrocatalytic reduction. Therefore in our study there are different ratios of Pd-Au NPs were investigated. Among them Pd-Au (0.8:0.2) probably purely heterogeneous-catalytic decomposition of hydrogen peroxide successfully competes with the electro-reduction reaction, thus lowering the effective concentration of peroxide. This might be due to the availability of numerous different catalytically active sites formed on the surface of Pd-Au (0.8:0.2) modified glassy carbon. For the remaining ratios of modifiers the absences of such a strong impact of the heterogeneous catalytic pathway on the overall process<sup>15-18</sup>.

**Table I:** Estimation of the area of deposited palladium-gold nanoparticles in 5 s deposition on GCE

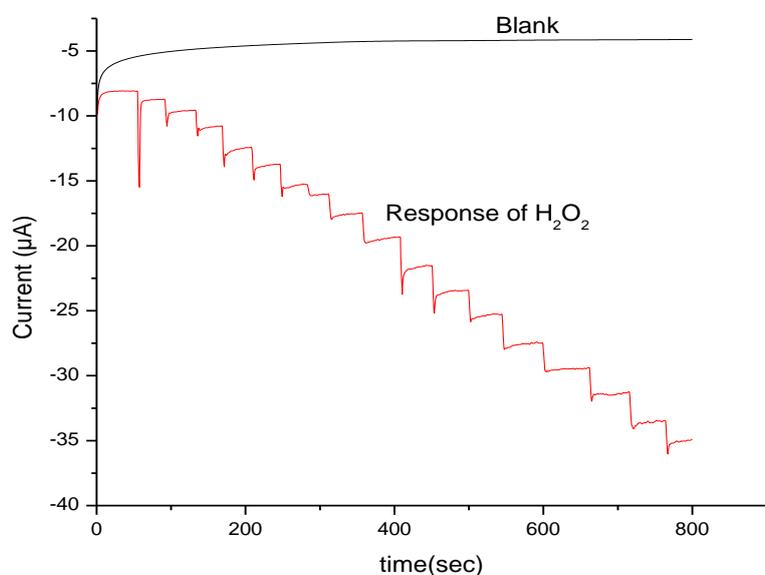
Pd-Au mole ratios	Pd surface area (cm <sup>2</sup> ) 1cm <sup>2</sup> = 424 μc <sup>17</sup>	Au surface area (cm <sup>2</sup> ) 1cm <sup>2</sup> = 400 μc <sup>17</sup>
0:1	-	0.028
1:0	0.056	-
0.9:0.1	0.166	0.012
0.8:0.2	0.062	0.008
0.6:0.4	0.057	0.040
0.4:0.6	0.017	0.110
0.2:0.8	0.028	0.087
0.1:0.9	0.016	0.050
0.3:0.7	0.031	0.022
0.7:0.3	0.047	0.019
1:1	0.034	0.012

The area of palladium-gold deposited can be quantified by formation of a palladium oxide and gold oxide layer in 1 mol/L H<sub>2</sub>SO<sub>4</sub>. Estimated from the charge related to the reduction peak of the surface oxide formation, that is for the reduction of palladium- gold surface oxides from electrochemically deposited palladium-gold nanoparticles on surface of GCE. The measurement was performed using linear scan voltammetry (LSV) where oxide formation was achieved by maintaining the potential of the electrode at 1.1 V for 5 s and scanning in a negative direction until -0.2 V with a scan rate of 0.5 Vs<sup>-1</sup> for palladium and 1.4 V for 5 s and scanning in a negative direction until -0.2 V with a scan rate of 0.5 Vs<sup>-1</sup> for gold oxide formation. Bimetallic catalysts can have considerably more favorable activities, selectivity, and/or deactivation properties than monometallic catalysts<sup>19</sup> and the combination of gold and Pd is no exception. Gold is traditionally considered to be a catalytically inert material<sup>20</sup> but it is known to enhance the catalytic properties of Pd for various chemical reactions. How Au promotes Pd

catalysis is not exactly known for most chemical reactions, and it remains a subject of considerable academic and industrial interest<sup>21-24</sup>.

### 3.13. Amperometric determination of $H_2O_2$ (0.8: 0.2)

The amperometric response of the hydrogen peroxide biosensor was investigated by successively adding  $H_2O_2$  to a continuous stirring PBS solution under the optimized conditions. The typical current–time curve of the biosensor is shown in figure 11. The response current increases with increasing concentration of  $H_2O_2$  as illustrated in figure 11. In this work, the applied potential of  $-0.250$  V was chosen. As shown in the figures a well-defined response was observed during the successive additions of  $H_2O_2$ . The current-time response of the Pd-Au NPs modified GCE for successive additions of  $H_2O_2$  increases stepwise with each additions of hydrogen peroxide.

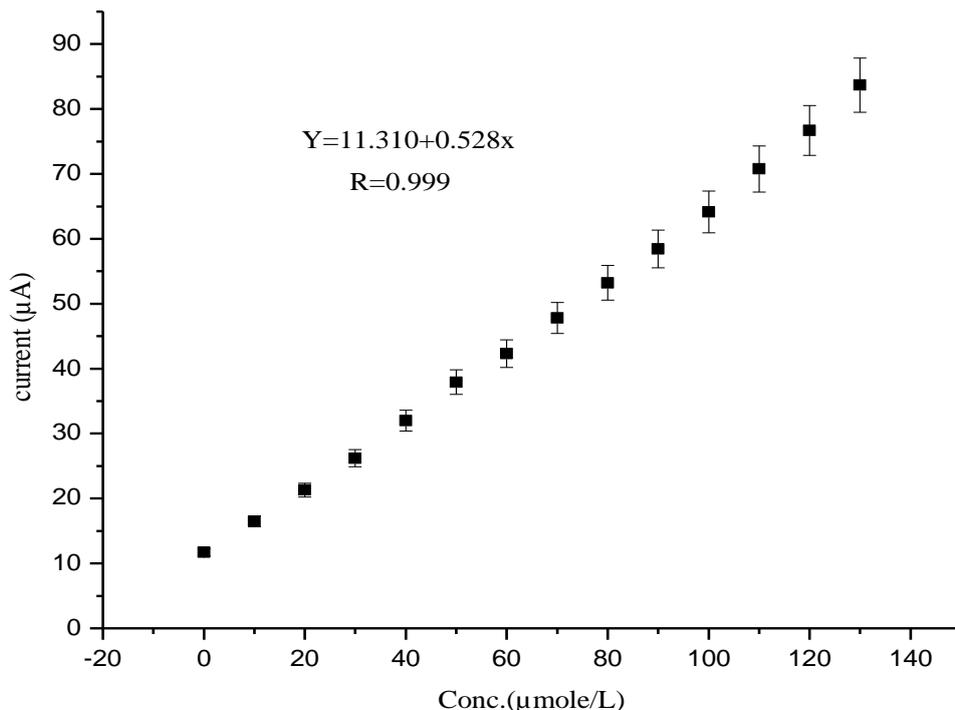


**Figure 11:** A current-time response curve for successive additions of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0, 130.0, ... and 170.0  $\times 10^{-6}$  mol/L  $H_2O_2$  for glassy carbon electrode modified with Pd-Au (0.8:0.2) NPs in 10.0 mL 0.1 mol/L pH 7.6 PBS with stirring 550 revolution per second.

The calibration curve shows that the response of Pd-Au NPs modified GCE to  $H_2O_2$  has a linear relationship in the range  $5.0 \times 10^{-6}$  to  $170.0 \times 10^{-6}$  mol/L with the regression equation:

$$y = 0.528x + 11.310, \text{ where } R = 0.999$$

The current response of the Pd-Au NPs modified GCE at  $-0.250$  V (vsAg/AgCl) in 0.1 mol/L PBS (pH 7.6) showed a linear response to hydrogen peroxide concentration ranging from  $5.0 \times 10^{-6}$  to  $170.0 \times 10^{-6}$  mol/L with detection limit of  $3.8 \times 10^{-7}$  mol/L.



**Figure 12:** Calibration curve for Pd-Au (0.8:0.2) NPs mole ratio modified GCE for determination of H<sub>2</sub>O<sub>2</sub>.

3.14. Real sample analysis

In order to demonstrate the applicability of the proposed modified electrode for real sample analysis mouth wash which contains H<sub>2</sub>O<sub>2</sub> solution was used. The concentration of H<sub>2</sub>O<sub>2</sub> in the mouth wash was determined by titration was 0.38 ± 0.00015 mol/L. This 0.38 mol/L solution was then diluted to 10,000 fold and spiked in to 0.01 mol/L H<sub>2</sub>O<sub>2</sub> standard solution. The result was shown below in table 2; therefore, the proposed modified electrode is applicable for less concentrated samples.

**Table II:** Concentration of H<sub>2</sub>O<sub>2</sub> detected in real sample

Concentration of H <sub>2</sub> O <sub>2</sub> Detected	Au-NPs modified	P-NPs modified	Pd-Au (0.8:0.2) modified
	Not detected	Not detected	0.414 ± 0.00011mol/L

From the above table we can observe the enhanced ability of the mixed Pd-Au (0.8:0.2) NPs modified GCE ability to detect H<sub>2</sub>O<sub>2</sub> from real sample which is difficult to detect with Pd and Au-NPs modified GCE with highly diluted concentration.

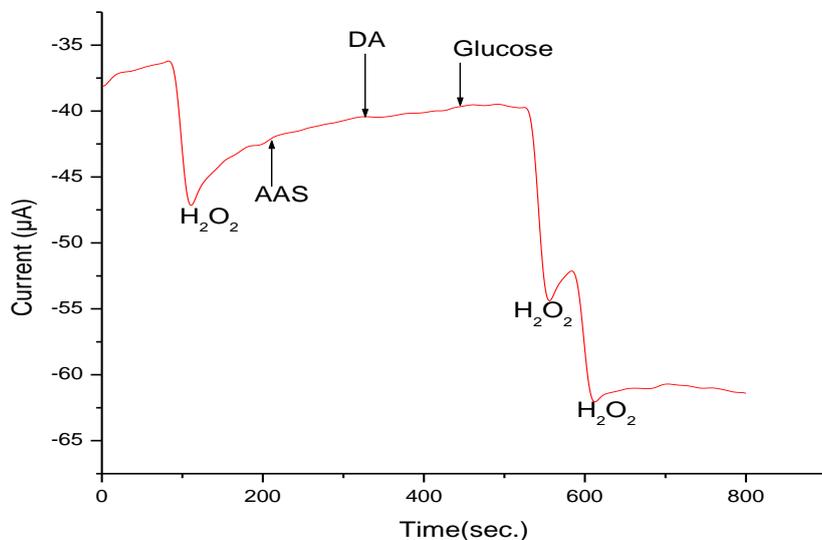
3.15. Reproducibility and stability of the modified electrode

The reproducibility and stability of the Pd-Au (0.8:0.2) atomic ratio modified electrodes have been investigated by examining the CV responses in 0.1 mol/L PBS pH 7.6 containing 0.003 mol/L  $\text{H}_2\text{O}_2$ . Investigation indicated that the peak current and peak potential of the modified electrode have remained nearly unchanged, and the amount of degradation after many times trial with scan rate of  $0.5 \text{ V s}^{-1}$  was less than 5 % that is the relative standard deviation of 2.9 % was observed for six repeated measurements of 0.003 mol/L  $\text{H}_2\text{O}_2$  with the same electrode.

The storage stability of the Pd-Au (0.8:0.2) atomic ratio modified electrode was also investigated by monitoring its response 0.003 mol/L  $\text{H}_2\text{O}_2$ , and it remained about 95 % of its original sensitivity after 15 days and it remained about 89 % of its original sensitivity after 30 days this Showing the proposed biosensor possesses a good stability and it is almost near to the result reported<sup>79</sup>. Generally these investigation indicated that the electrochemical behavior of the Pd-Au (0.8:0.2) atomic ratio modifier reproducibility and stability while determination of  $\text{H}_2\text{O}_2$ .

To explain further about the storage stability of the proposed modified electrode, when not in use, the electrode was stored in 0.1 mol/L PBS in refrigerator. The response to 0.003 mol/L  $\text{H}_2\text{O}_2$  was tested continuously. During the first 3 days, day by day no change was observed, and after other 3 days the response current has about 0.65 % decreases and in addition the next 1 week and 2 weeks the current response decreases about 2.2 % and 2.75 % respectively of its initial response. Then after 3 weeks and 4 weeks decrease of current response was 2.96 %, and 3.04 respectively of its 2<sup>nd</sup> week response. This tells us the highly stability of the modified electrode.

### 3.16. Interference free determination of $\text{H}_2\text{O}_2$ by modified electrode



**Figure 13:** Amperometric response of the hydrogen peroxide upon addition of 0.01 mol/L  $\text{H}_2\text{O}_2$ , 0.00015 mol/L ascorbic acid (AA), 0.000005 mol/L dopamine (DA), 0.005 mol/L glucose, and 0.01 mol/L  $\text{H}_2\text{O}_2$  at GCE modified with Pd-Au (0.8:0.2) NPs in 0.1 mol/L PBS pH 7.6 with stirring 550 revolution per second no interference effect was observed.

As interference study is important part in the determination  $H_2O_2$  the effect of common interfering electroactive substances such as ascorbic acid (AA), dopamine (DA) and glucose were assessed by making the potential window only specific for determination  $H_2O_2$  that is the expected interferences never show reduction current response with the selected potential and above figure 13 shows that the amperometric response for the injection of  $H_2O_2$  was free from the expected interference such as AAS, DA, and glucose. From this information the current response is due to reduction of  $H_2O_2$  and this due to the specificity of potential window for  $H_2O_2$  and selectivity of the sensor for  $H_2O_2$ .

#### 4. Conclusion

In this study, the electro-catalytic reduction of hydrogen peroxide on a glassy carbon electrode modified with Pd-Au nanoparticles atomic ratio co-deposit as compared to Pd nanoparticles, and Au nanoparticles electrode was investigated with optimizing the experimental parameters such as potential, deposition time, and pH of supporting electrolyte at which maximum reduction current was obtained. Among these 11 developed sensors, Pd-Au (0.8:0.2) atomic ratio exhibits high sensitivity and fast response to  $H_2O_2$  determination. Bimetallic NPs composed of Au and Pd has been successfully synthesized and the catalytic properties of Pd-Au NPs gave the enhanced electrochemical responses for hydrogen peroxide. This improved analytical performance makes Pd-Au NPs modified GCE promising for an amperometric sensor for hydrogen peroxide and the expected interferences interfering was controlled while electrochemical determination of  $H_2O_2$  by making the potential window specific and selective for  $H_2O_2$ . Due to electro catalytic activity and significantly lower detection limit of this modified electrode favorable as compared to Pd-NPs, Au-NPs, and other enzyme modified electrode employed as hydrogen peroxide sensors. In addition to this, the modification procedure is less expensive and more convenient. The analytical performance of the modified electrode indicates that it can be used as sensitive amperometric detector for lower concentration detection of hydrogen peroxide in real samples.

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