

JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY



ELECTROCHEMICAL DETERMINATION OF HYDROGEN PEROXIDE (H₂O₂)
AT GLASSY CARBON ELECTRODE MODIFIED WITH A PALLADIUM-GOLD
CO-DEPOSITE NANOPARTICLES

BY: BELAY GEZAHEGN

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES, JIMMA
UNIVERSITY, DEPARTMENT OF CHEMISTRY, IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN
CHEMISTRY (ANALYTICAL CHEMISTRY)

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List of Abbreviation

A	Ampere
AFM	Atomic Force Microscopy
CA	Chronoamperometry
CV	Cyclic Voltammetry
GCE	Glassy Carbon Electrode
HRP	Horseradish Peroxidases
LOD	Limit of Detection
LSV	Linear Sweep Voltammetry
NPs	Nanoparticles
PBS	Phosphate Buffer Saline
s	Seconds
SPM	Scanning Probe Microscopy
STM	Scanning Tunneling Microscopy
V	Volt
μ	Micro

Abstract

Electrochemical methods using 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_2O_2 . The optimization of the experimental conditions such as deposition potential and deposition time of the co-deposit, pH for electroanalysis of H_2O_2 and reduction potential for H_2O_2 at modified electrode 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 3.8×10^{-7} mol/L under the optimized conditions. The fabricated nanoparticles were applied for analysis of H_2O_2 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.

1. Introduction

Electroanalysis is a technique utilized to determine species in solution, especially aqueous solution, both quantitatively and qualitatively. The advantage of electroanalytical techniques over other detection methods such as chromatography, luminescence and spectroscopy is the low cost requirements and the ease of use, accuracy and reliability.

A variety of techniques are available to the researcher to study the electrochemistry of electroactive species in solution¹. These processes can be influenced by several factors, including the nature of the analyte under investigation, the type of electrode and the choice of electrolyte¹.

The size and morphology of the electrode and the fabrication method used can be very influential on the voltammetric response of the system². Compared with other electrode concepts in electrochemistry, the distinguished features 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 and 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^{3,4}. 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.

Nowadays many types of monometallic and bimetallic nano particles (NPs) of different sizes and compositions are available for electrode surface modification, which facilitate their application in electroanalysis, bringing important advantages: A) their immobilization on electrode surfaces generates a roughened conductive high-surface area interface that enables the sensitive electrochemical detection of molecular and bimolecular analytes: B) NPs act as effective labels for the amplified electrochemical analysis of the respective analytes: C) the architectures with pre-designed and controlled electrochemical functions^{5,6}.

In recent years, the use of nanoscale materials has been extensively studied⁶, particularly with respect to metallic NPs⁶. Underpinning the significance of nanoparticulate materials in electroanalysis 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 properties⁶.

The use of NPs also provides control over the local microenvironment⁶. This can be highly advantageous when incorporating sensitive or biological materials into a system. These unique properties make nanomaterials ideally suited for electroanalytical applications. Enhanced convergent mass transport to nanoelectrodes facilitates the study of faster electroanalytical processes⁶.

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 catalysis⁷. A few commonly used metals for NPs are gold⁷, silver⁵ and platinum⁸. 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 palladium⁸, ruthenium¹¹, nickel, and copper⁷. Furthermore, this study was investigated for fabrication of more electro active sensor of H₂O₂ 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 H₂O₂⁸⁻¹², 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 H₂O₂. It has also been known that the amount of metal nanoparticles depends on the length of deposition time, therefore by performing the deposition time optimizing and minimizing the high overpotential the more electroactive fabrication going to be done.

1.1. Theoretical Background

1.1.1. Electrochemical Sensors

An electrochemical sensor can be defined as a device that provides continuous information about its environment. Ideally, a chemical sensor provides a certain type of response directly related to the quantity of a specific chemical species. All chemical sensors consist of a transducer, which transforms the response into a detectable signal, and a chemically selective layer, which isolates the response of the analyte from its immediate environment. Electrochemical sensors provide important applications in the fields of clinical, industrial, environmental and agricultural analysis¹³. The applied current or potential for electrochemical sensors may vary according to the mode of operation, and the selection of the mode is often intended to enhance the sensitivity and selectivity of a particular sensor¹⁴.

1.1.2. Definition of Nanoparticles

The term “nano” is derived from the Greek word for “dwarf”, “nanos”. This etymology, and its placement on the metric scale ($1\text{ nm}=10^{-9}\text{ m}$), which is not visible to the naked eye, beyond the normal limits of our observation¹⁵. Those properties of nanoparticles which differ from the properties of the bulk material are of particular interest. Otherwise, the importance of nanoparticles would be limited to the aspects of savings in materials and expenses, and of miniaturization, which are indeed of the greatest importance commercially¹⁵.

1.1.3. The Reasons for Using Nanoparticles

The determining factor for the use of nanoparticles in catalysis is the large ratio of surface area to volume, with a large number of catalytically active centers at the surface and relatively few inactive inner atoms. In addition to the optimization of known catalysts by transfer to the nanoscale, increasing efficiency and selectivity is the subject of the current research¹⁵. But novel materials and considerable progress in analytical characterization have also contributed to a better understanding of the interactions between particle properties and catalytic activity^{15,16}.

1.1.4. Nanoelectrochemistry

Nanoelectrochemistry is a branch of electrochemistry that investigates the electrical and electrochemical properties of materials at the nanometer size regime. Nanoelectrochemistry plays significant role in the fabrication of various sensors and devices for detecting molecules at very low concentrations¹⁷. It also combines characteristics of electrochemistry (e.g., simplicity, speed, high selectivity and high sensitivity) with the unique properties of nano particles (e.g., electronic, optical, magnetic and catalytic)¹⁷.

1.1.5. Modifying of the Electrode Surface

Modifying the surface of electrodes to provide some control over how the electrode interacts with its environment has been one of the most active areas of research interest in electrochemistry. Whereas once the performance of an electrode was limited to the solution it was placed into, the material from which the electrode was made and the potential applied to the surface, the ability to chemically modify electrodes has provided a powerful route to tuning their performance. This has been particularly important to electroanalytical chemistry and modification has provided routes to providing selectivity, resisting fouling, concentrating species, improving electrocatalytic properties and limiting access of interferences in a complex sample such as a biological fluid, but has also had major impact for research into energy conversion and storage, corrosion protection, molecular electronics electrochromic devices and fundamental research into phenomena that influence electrochemical processes¹⁸.

1.1.6. Electrodeposition of Metal Nanoparticles

The fabrication of metal nanoparticle on glassy carbon electrodes by electrochemical techniques is advantageous because it is a single step process involving reduction of the metal salt at an applied potential and therefore, is a convenient, fast and low-cost method for the preparation of metal nanoparticles on large areas of conductive substrates. Furthermore, electrodeposited metal nanoparticle electrodes can be easily manufactured under ambient conditions on an industrial scale by simple modification of commercial electroplating operations. Electrochemical deposition procedures are also advantageous because they allow the properties of the deposited materials to be easily controlled in order to optimize the electrode for the desired reaction. The nucleation and growth mechanism and hence, the size and density of metal particles can be

controlled simply by varying the deposition conditions such as electrolyte composition, deposition time and/or overpotential^{17,19-21}.

1.1.7. Electrochemical Deposition Methods of Metal Nanoparticles

The surface structuring of electrodes with nanoscale features depends on the method by which electrochemical deposition of metals performed.

Therefore the two main electrochemical deposition approaches of metal nanoparticles in surface nanostructuring using electrochemistry are:

- a) **Random electrochemical deposition of metal nanoparticles.** This is one of the electrochemical depositions methods of metal nanoparticles on a GCE surface which can takes place without controlling the distribution of metal nanoparticles on a GCE surface. Means that this type of metal nanoparticles deposition never needs instruments in order to locate the electrochemical deposition process. Therefore glassy carbon electrodes offer a practical advantage for creating these kinds of structures as a result of the availability of random surface defect that can serve as nucleation centers during the electrochemical reduction of metal ions^{17, 18}.
- b) **Defined electrochemical nanostructuring with metallic nanoparticles.** This is the second electrochemical depositions methods of metal nanoparticles on a GCE surface which can takes place with controlling the distribution of metal nanoparticles on a GCE surface. This approach requires tools in order to locate the electrochemical deposition process at the substrate/electrolyte interface. Scanning probe microscopy (SPM) techniques have been used for this purpose. Scanning tunneling microscopy (STM) tips or metalized atomic force microscopy (AFM) cantilevers have also been used as miniaturized electrodes to deposit metal nanoclusters or to create nano-defects on a substrate^{17, 22}.

1.2. Statement of the Problem

The electrochemical behavior and applications of nanoparticles have received increasing attention. The high ratio of surface atoms with free valences to the cluster of total atoms gives rise to high catalytic activation, which has been used in electrochemical catalytic reactions. Thus, a description of the electroanalytical properties of several metal nanoparticles was done for electrochemical analysis of hydrogen peroxide in different samples^{5,12,13}.

Non electrochemical studies were employed for hydrogen peroxide analysis in different analytes. Their drawbacks are high overpotential, less selectivity, less sensitivity, less efficiency with low concentration of analytes. Therefore, the use of electrochemical technique is due to the ability to determine the analyte with high sensitivity, selectivity, and efficiency of sensor with low concentration of analytes.

Therefore this study may answer for the following questions:

- ❖ Is palladium modified glassy carbon (GC) or palladium gold co-deposit modified GC have higher sensitivity for determination of hydrogen peroxide (H_2O_2)?
- ❖ Is gold modified glassy carbon (GC) or palladium gold co-deposit modified GC have higher sensitivity for determination of hydrogen peroxide (H_2O_2)?
- ❖ What will be the responsible reason for having different response with different electrodes?
- ❖ Among Pd-Au co-deposit modified electrode which ratios of modification are more sensitive?

2. Literature Review

2.1. Chemistry of Palladium-Gold

Palladium based bimetallic catalysts have been developed since many years to increase the selectivity and sensitivity of sensors²³. The promotion effect of Au on Pd catalytic activity is a well-known²⁴ phenomenon for different reactions, including the commercial production of vinyl acetate. Pd-Au and Pt-Au catalysts have been studied for polyol (sorbitol and glycerol) oxidation²⁴: the addition of Au to Pd or Pt catalysts does not only improve catalytic activity and selectivity for the polyol oxidation but also enhances the resistance to poisoning. On the other hand, it has been shown recently that gold-based nanoparticles are promising electrocatalysts for application in fuel cells^{24, 25-27}.

In addition to the above statements, the Pd–Au bimetallic catalysts often display enhanced catalytic activities and selectivities compared with Pd-alone catalysts. This enhancement is often caused by two alloy effects, *i.e.*, ensemble and ligand effects. The ensemble effect is a dilution of surface Pd by Au. With increasing surface Au coverage, contiguous Pd ensembles disappear and isolated Pd ensembles form. The ligand effects are electronic perturbation of Pd by Au via direct charge transfer or by affecting bond lengths, the ligand effects cause the Pd d band to be more filled, moving the d-band center away from the Fermi level²⁸.

They also exhibit enhanced catalytic performances in terms of activity, selectivity, and stability, compared to the separate component²⁶. The catalytic electrocatalytic activities could strongly depend on the size and shape of the metal nanoparticles. Therefore, synthesis of bimetallic nanoparticles that could exhibit well-controlled shapes, sizes, chemical composition, and structure has been explored in order to enhance their performances^{24, 29}. The promotion of the catalytic properties is explained on the basis of an enhancement of the electronic properties which, in terms of electronic configuration and electronegativity, is different from either pure Au or pure Pd³⁰. In alloyed NPs, the key parameter that dictating catalytic activity is the Au:Pd atomic ratio and the chemical order at the surface. In NPs other than those with core-shell structures³¹, Au-Pd NPs present a complete disordered atomic arrangement of Au and Pd atoms³¹. Adding small amount of gold to palladium and forming highly uniform nanoparticle

cores make the platinum monolayer electrocatalyst significantly tolerant and very promising for the automotive application of fuel cells³².

2.2. Electrocatalysis of Hydrogen Peroxide

Because of the importance of H₂O₂ in several fields such as environmental, industrial, food, clinical and biochemical analysis, the development of reliable, rapid and economic methods of sensing H₂O₂ is of great significance for numerous processes and has become a subject of study for decades. Many analytical methods have been reported for the determination of H₂O₂ such as titrimetry³³, fluorimetry³⁴, chemiluminescence³⁵, spectrophotometry³⁶ and electrochemistry^{37, 38}. Among these, electrochemical detection is one of the promising approaches to achieve accurate, specific, economic and rapid H₂O₂ monitoring. The advantages of using electrochemical method are low detection limits, large dynamic concentration range, good selectivity, rapid response time and inherent miniaturization and portability. However, these features depend on the use of mediators capable of recognizing the analyte in complex samples. Several chemical compounds have been attached onto the electrode to minimize the energy required in the electrochemical reduction of hydrogen peroxide³⁹⁻⁴². Therefore the electrochemical method needs chemical modification of surfaces of electrodes and this chemically surface modification can be divided in two main groups: nonenzymatic surface modification (metal nanoparticles or functionalized nanoparticles act as sensing phase) and enzymatic surface modification (enzyme-modified metal nanoparticles act as sensing phase, where nanoparticles work as mediators)⁴³.

2.2.1. Enzymatic Determination of Hydrogen Peroxide

Enzymatic determination of hydrogen peroxide is detecting H₂O₂ by using enzyme-modified electrode or enzyme-modified metal nanoparticles electrode act as sensing phase, where nanoparticles work as mediators⁴³. Studies were usually investigated for amperometric determination of H₂O₂ at platinum surfaces⁴⁴. And the great shortcoming observed in this approach was the high over potential needed for H₂O₂ oxidation that is 0.7 V vs. Ag/AgCl) at which many electroactive species such as ascorbic acid, uric acid could also be oxidized to give interfering signals. For this drawback the most common ways to overcome the problem, has been the use of an enzyme, namely horseradish peroxidase (HRP), a prototypical heme protein

peroxidase, which catalyses the reduction of H_2O_2 and, due to its peculiar structure, allows the direct electron transfer between its active site and the electrode surface^{45,46}. Using this system, the electrochemical detection of hydrogen peroxide can be performed at much lower potentials (-0.1 to 0 V vs. Ag/AgCl) where the responses from the enzyme-catalyzed reaction are based on the reduction of the enzyme active centre and not the direct reduction of hydrogen peroxide⁴⁷. Though direct electrical communication between HRP and common electrodes is observed, generally, it is a slow process⁴⁸. A faster electron transfer could be achieved by means of mediators⁴⁹ and, in reagent less biosensor systems, by means of conducting polymers which can transfer charges from the electrode to the enzyme active site more efficiently⁵⁰. Despite the advantages of good sensitivity and accuracy, biosensors suffer from important shortcomings such as high cost, low stability and limited binding of the enzyme to solid surfaces⁵¹.

2.2.2. Metal NPs Based Determination of Hydrogen Peroxide

To overcome the above drawback of only enzyme modified electrodes, studies was investigated electrochemical biosensors based on oxidase-modified electrodes, which react with bio-substrates and yield H_2O_2 . Electrical contacting of redox-enzymes with electrodes is a key process in the construction of enzyme electrodes. While enzymes usually lack direct electrical communication with electrodes due to the fact that the active centers of enzymes are surrounded by considerably thick insulating protein shells, and the electron transfer between electrodes and the active centers are blocked, the conductivity properties of NPs, mostly metal nanoparticles at nanoscale dimensions made them suitable for enhancing the electron transfer between the active centers of enzymes and electrodes acting as electron transfer “mediators” or “electrical wires”⁵²

An elaborate review about the fundamentals and applications of peroxidase-modified electrodes shown⁵³. In earlier days, amperometric detection of H_2O_2 was usually performed at either platinum (Pt) or platinized surface sensor^{54 55}. Yet, the trend has been employing chemically modified electrodes to detect H_2O_2 since two decades ago. And as it was reported that a glassy carbon (GC) electrode modified by a thin film composed of palladium and iridium could catalyze the reduction of H_2O_2 ⁵⁶. In contrast to the reduction of H_2O_2 , modified a GC electrode by a thin film of oxymanganese species, which catalyzed the oxidation of H_2O_2 ⁵⁷. Also, monitored a

fermentation process by detecting the oxidation of H_2O_2 at a GC electrode modified by an oxycobalt film⁵⁸.

Nevertheless, it is well known that carbon nanotubes (CNTs) are suitable materials for electrode modification and support in biosensor applications because of the high accessible surface area, low electrical resistance, extremely high mechanical strength and stiffness, outstanding charge-transport characteristics, high chemical stability, and excellent biocompatibility⁵⁹⁻⁶³.

The immobilization of different electron transfer mediators on the surface of various electrode materials has been attracting increasing interest for applications of chemically modified electrodes in the fields of electrocatalysis and electroanalysis. Appearance of nanomaterials may provide a good chance for developing simple and efficient methods to fabricate modified electrodes due to their peculiar properties, and various nanomaterials have been used to incorporate electron transfer mediators for constructing electrochemical biosensors, such as carbon nanotubes (CNTs), carbon nanofiber, nanogold and nano TiO_2 ⁶⁴ etc.

2.2.3. Non-Enzymatic Determination of Hydrogen Peroxide

Non-enzymatic determination of hydrogen peroxide is detecting H_2O_2 by using electrode modified with metal nanoparticles or functionalized nanoparticles act as sensing phase⁴². Metal nanoparticles have a large surface to volume ratio which contributes to the probability of electrocatalytic activity^{65, 66}. The most common methods used to prepare metal nanoparticles electrode for the direct determination of H_2O_2 have involved electrochemical deposition, assembly of functionalized colloidal metal particles onto a solid substrate or dispersion of pre-formed particles within a carbon paste mixture⁶⁷.

The electrochemical methods have the advantages of easy for preparation, fast detection, low consumption, and high selectivity and sensitivity⁶⁸. But the direct electrochemical reduction of H_2O_2 at ordinary solid electrodes is a slow electrode process that requires a large overpotential, which is the major barrier for determination of H_2O_2 by electrochemical methods. Direct reduction of H_2O_2 at several metal-modified (nanostructured) electrodes, including silver⁶⁹, gold⁷⁰, platinum⁷¹, palladium⁷², and cobalt porphyrin⁷³ have been investigated.

Additional investigation were also reported that metal nanoparticles exhibit efficient and preferential catalytic activity towards H_2O_2 and detection has been made via H_2O_2 oxidation or reduction at reduced over potentials⁷⁴⁻⁷⁶. The detection of hydrogen peroxide was successfully observed in by using Au-NPs electrodeposited onto a GCE enabled the detection of H_2O_2 by reduction at -0.68 V (vs. saturated calomel electrode(SCE)) as no response was observed at the glassy carbon substrate⁷⁵.

2.3. Hypothesis

The use of precious metal in electroanalytical chemistry has been explored by several research groups because these metals can modify bare glassy carbon electrode surface electrochemically in a simple and controlled way. Therefore, electrochemical modifications of glassy carbon electrode with metal nanoparticles minimize the high overpotential which makes the electrode sensitive to different interferences from many electroactive species.

Therefore our proposed electrode may be able to minimize overpotential by minimizing the nanoparticles size, having high selectivity, high sensitivity and low detection limit to H_2O_2 analysis.

3. Objectives

3.1. General Objective:

- ❖ To develop sensitive electroanalytical method for the determination of hydrogen peroxide based on mixed metal substrate.

3.2. Specific Objectives:

- ❖ Investigation of electrochemical behavior of H_2O_2 at Pd-Au modified electrodes.
- ❖ To optimize electrochemical parameters for modification of GC electrode with Pd-Au co-deposit nanoparticles.
- ❖ To optimize electroanalytical parameters for determination of H_2O_2 at Pd-Au co-deposit nanoparticles.
- ❖ To determine H_2O_2 under optimized condition at Pd-Au modified electrodes.
- ❖ To compare the performance of GCE modified by Pd-Au co-deposit NPs with that of Pd-NPs and Au-NPs modified GC electrodes.

4. Experimental Section

4.1. Chemicals and Reagents

Palladium dichloride (PdCl_2 , 99.9%, Aldrich), potassium tetrachloroaurate (III) (KAuCl_4 , 98%, Aldrich), sodium perchlorate (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 (NaCl , 99.8% Riedel-de Haen), hydrochloric acid (HCl, 37%, Riedel-de Haen), potassium hydrogen phosphate (K_2HPO_4 , 98 %, FINKEM), potassium dihydrogen phosphate (KH_2PO_4 , 99%, NICE), potassium iodide (KI, 99%, Riedel-de Haen), sulfuric acid (H_2SO_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 (H_2O_2 , 30%, Aldrich), phosphoric acid (H_3PO_4 , 98.5 %, FINKEM). Distilled water was used to prepare all aqueous electrolyte solutions throughout the study.

4.2. 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.

4.3. Analytical Methods

4.3.1. Palladium Deposition Bath Preparation

A Pd deposition bath consisting of 0.0075 mol/L PdCl_2 in citrate buffer prepared by dissolving 66.50 mg of 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 of pH was adjusted to 3 by addition of diluted KOH (0.1 mol/L)⁷⁷.

4.3.2. Gold Deposition Bath Preparation

Gold deposition bath, containing a 0.05 mol/L KAuCl_4 , was dissolved in 0.5 mol/L H_2SO_4 solution. To prepare the deposition bath, 0.028 mg of KAuCl_4 was added to 1.5 mL of 0.5 mol/L H_2SO_4 solutions. For deposition bath 0.001 mol/L KAuCl_4 was prepared by diluting 0.1 mL of 0.05 mol/L KAuCl_4 stock solutions in 5 mL of 0.5 mol/L H_2SO_4 ¹⁸. The reason why citrate buffer not used to prepare Au deposition is, because of citrate buffer can reduce gold.

4.3.3. Pd-Au Co-deposition Bath Preparation

To prepare the Pd-Au co-deposit bath identifying the solvent which can dissolve both salts of palladium and gold completely is the first procedure because if we use H_2SO_4 PdCl_2 is slightly soluble in diluted H_2SO_4 and if we use citrate buffer it can reduce gold. Therefore to solve this problem HNO_3 was used for the preparation of Pd-Au co-deposit bath because it can dissolve both salts completely.

A 0.05 M PdCl_2 of palladium and 0.05 mol/L KAuCl_4 of gold stock solutions were prepared by dissolving both salts in 0.5 mol/L HNO_3 solution separately. To prepare 0.05 mol/L KAuCl_4 stock solution of gold 0.028 mg of KAuCl_4 was dissolved in 1.5 mL of 0.5 mol/L HNO_3 solution and for preparation of 0.05 mol/L PdCl_2 stock solution of palladium, 0.0133 mg of PdCl_2 was dissolved in 1.5 mL of 0.5 mol/L HNO_3 solution. Then, for deposition bath 0.001 mol/L KAuCl_4 was prepared by diluting 0.1 mL of 0.05 mol/L KAuCl_4 stock solution in 5 mL 0.5 mol/L HNO_3 and the same procedure was followed for preparation of palladium deposition bath with 0.001 mol/L PdCl_2 . After preparing 0.001 mol/L PdCl_2 and 0.001 mol/L KAuCl_4 solution from their salts by dissolving in 0.5 mol/L HNO_3 solution, different mole ratios of Pd-Au co-deposition bath prepared by taking appropriate volume for each ratios.

4.3.4. Standardization of Hydrogen Peroxide

Hydrogen peroxide was standardized by iodimetric titration⁷⁸. A stock standard solution containing H_2O_2 was prepared freshly each day. Working standard solutions of lower concentrations were prepared immediately before use.

4.4. Electrode Preparation

4.4.1. 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 NaClO₄ for at least five complete scans at 0.5 Vs⁻¹. The prepared electrodes were used immediately after mechanical polishing and electrochemical cleaning.

4.5. Metal Deposition

Palladium, Gold and Palladium-Gold electrode were prepared by potentiostatic deposition of PdCl₂, KAuCl₄ and PdCl₂ - KAuCl₄ mixture on cleaned GCE from Pd, Au and Pd-Au deposition baths respectively.

4.5.1. Au-NPs Modified GCE Fabrication

On a cleaned and electrochemically conditioned GCE, a solution of KAuCl₄ 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 further electrochemical studies.

4.5.2. Pd-NPs Modified GCE Fabrication

On a cleaned and electrochemically conditioned GCE, a solution of PdCl₂ 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 further electrochemical studies.

4.5.3. Pd-Au NPs Co-deposit Modified GCE Fabrication

From solutions of 0.001 mol/L PdCl₂ and 0.001 mol/L KAuCl₄ 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 of each mixture 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 further electrochemical studies.

5. Results and Discussion

5.1. 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 provides some basic information, the characteristic features of Au reduction and oxidation.

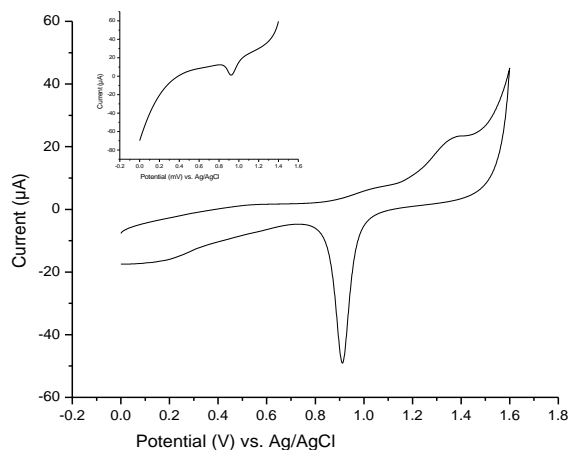


Figure 1: Cyclic voltammogram of Au deposition on a GC surface from a KAuCl_4 in 0.5 mol/L H_2SO_4 solution at scan rate 0.5 Vs^{-1} .

The resulting modified electrodes was characterized by using CV in H_2SO_4 0.5 mol/L. Figure 1 shows the electrochemical response of the gold NPs on GCE surface scanning between -0.2 and 1.4 V, for one complete electrodeposition scans. The anodic peaks at + 1.4 V were associated to Au oxidation and the backward scan, the peak at +0.921 V corresponded to the subsequent reduction of the oxides previously formed. To proof the presence of gold deposits on GCE surface the linear sweep voltammetry was performed to confirm by the formation of a gold oxide

layer followed by its reduction in the reverse scan, where the peak potential for reduction of gold oxide is + 0.921 V as shown in figure 1, inset.

5.2. Optimization of Experimental Parameters for Au-NPs Modified GCE

5.2.1. Influence of Deposition Time

The amount of metal particles on the surface of the electrode has great influence on the performance of the modified electrode. Therefore, the amount of metal particles on the electrode surface depends on the deposition time, which necessitated optimization of the deposition time.

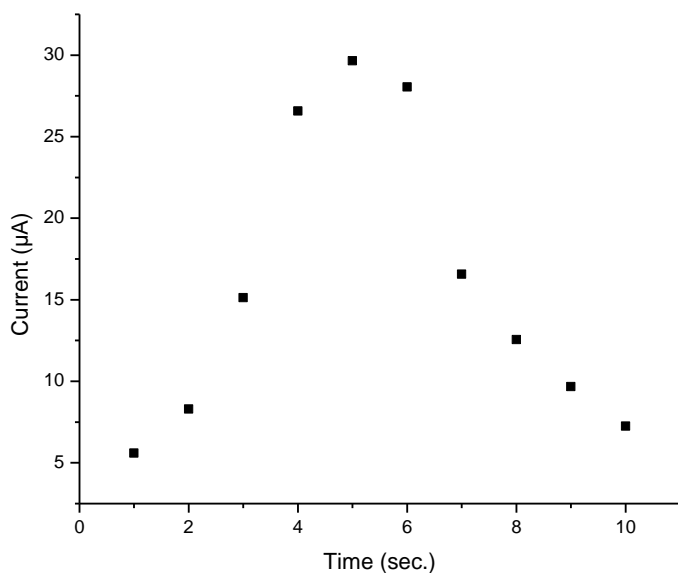


Figure 2: Plot of the response of Au-NPs modified GCE to various deposition times containing 0.04 mol/L H_2O_2 .

With increasing of Au-NP deposition time for modified GCE, the CV response increases up to 5 seconds Figure 3. Further increase of the deposition time led to decrease of the response. Because the smaller the particles the larger fractions of atoms at the surface and thus more reactive the surface and this obtained with low deposition time. As deposition time increase, the modification goes to film and smaller fractions of atoms at the surface and less reactive the surface to the analyte. Therefore, among the lower deposition time 1 s - 5 s the maximum

reduction current was obtained at 5 s deposition time. Therefore the optimized deposition time is 5 s and it applied for further experiment.

5.2.2. 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 range from 5.8 to 8.0, as shown in Figure 2 below.

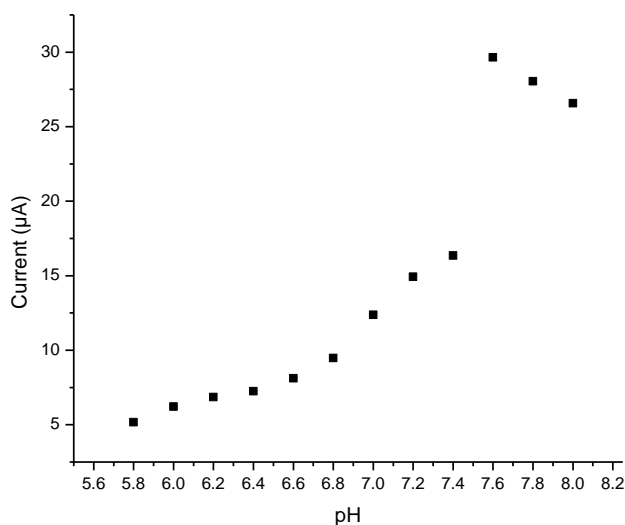


Figure 3: Plot of the response of Au-NPs modified GCE to various pH values containing 0.04 mol/L H_2O_2 .

Optimization of pH was performed by reading the CV maximum reduction current response to H_2O_2 . So with increasing of buffer pH, the amperometric response of the biosensor increased till pH 7.6. The further increase of buffer pH led to decrease of the response, indicating that the catalytic response was controlled by the fabricated Au-NPs activity in this region. Decrease in the response at high pH was possibly due to the decrease of fabricated Au-NPs activity to the analyte. So a buffer solution with pH 7.6 was selected for further experiments.

5.3. Electrochemical Behavior of Hydrogen Peroxide at Bare GCE

Figure 4 shows the electrochemical response of glassy carbon electrode to additions of hydrogen peroxide in phosphate buffer, with scan rate of 0.5 Vs^{-1} . The electrochemical responses were run over a range of scan rates but no significant voltammetric currents were observed. That means no obvious peak corresponding to the reduction and oxidation of hydrogen peroxide was observed at bare GCE and this result is lined with experimental result shown in⁷¹, where no reduction or oxidation peak for hydrogen peroxide was seen at bare GCE. This tells us the bare GCE is not able to detect H_2O_2 and it is inert to H_2O_2 .

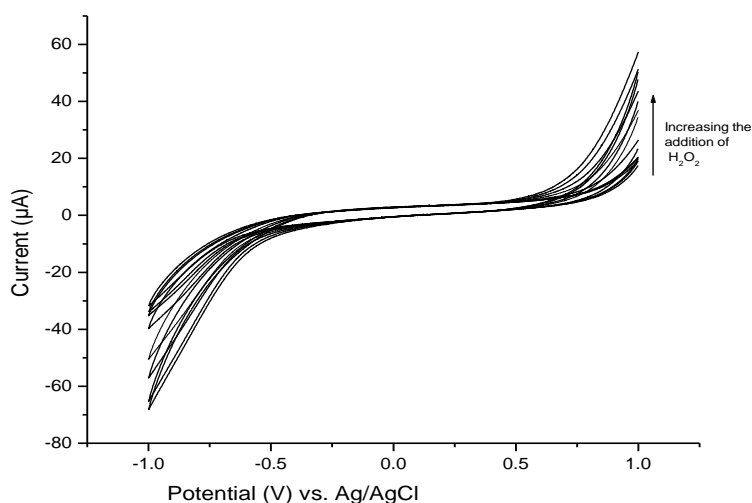


Figure 4: Cyclic voltammogram detailing the response of a GCE to the addition of H_2O_2 (0, 0.1, 0.5, 1, 5, 10, 15, 20 and $25 \times 10^{-3} \text{ mol/L}$). Run at a scan rate of 0.5 Vs^{-1} in PBS (0.1 mol/L).

5.4. Electrochemical Behavior of H₂O₂ at Au-NPs Modified on GCE

5.4.1. Cyclic Voltammetric Response of H₂O₂ Using Au-NPs Modified GCE

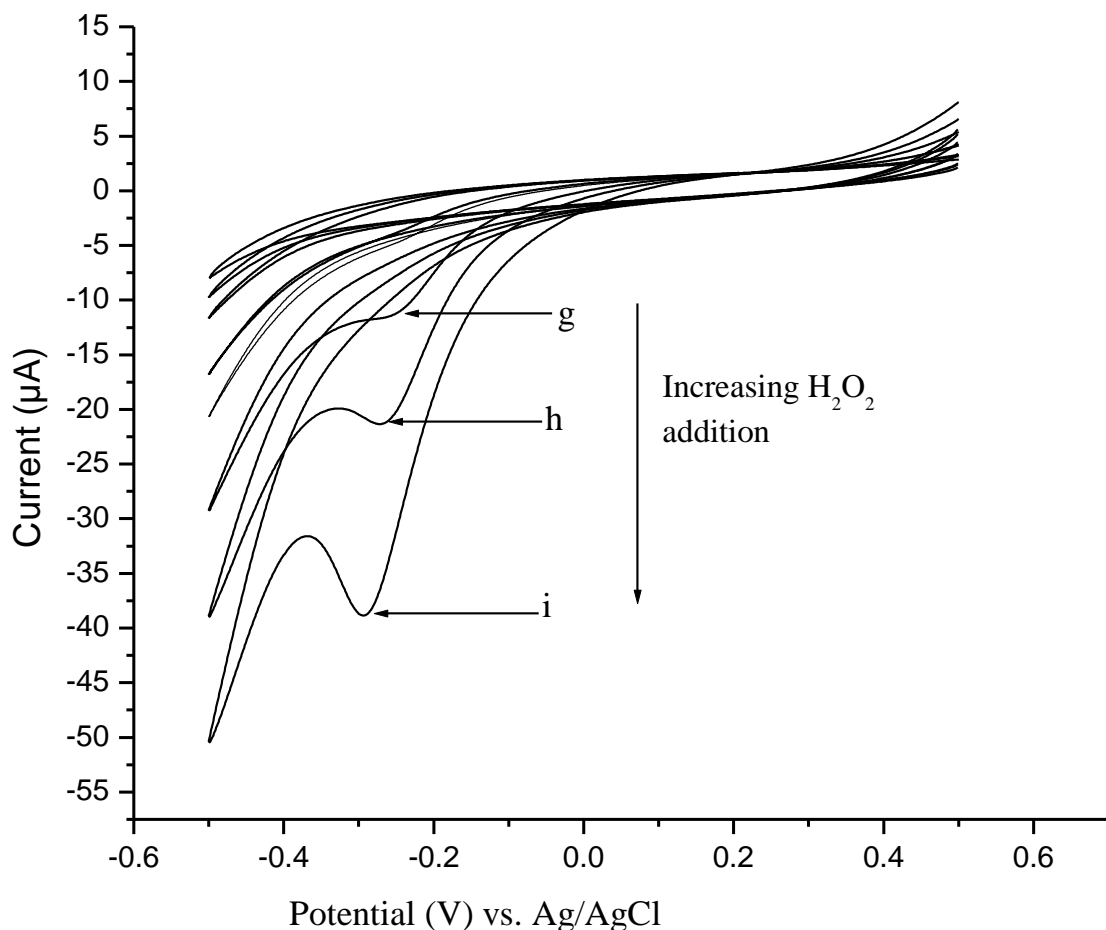


Figure 5: Cyclic voltammogram detailing the response of Au-NPs to the addition of H₂O₂ (a) 0, b) 0.1, c) 0.5, d) 1, e) 5, f) 10, g) 15, h) 20 and i) 25 × 10⁻³ mol/L). Run at a scan rate of 0.5 Vs⁻¹ in PBS (0.1 mol/L).

Figure 5 indicates the response of the GCE modified by electrochemically deposited gold to additions of hydrogen peroxide (H₂O₂). 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 H₂O₂ Au modified GCE gave a current response for reduction of H₂O₂ but higher current response was not observed.

5.4.2. Amperometric Determination of H₂O₂ at Au-NPs

Amperometric method was employed for determination of H₂O₂ at much lower concentrations than cyclic voltammetry. Figure 6 displays the current-time response of the Au-NPs modified GCE with successive injection of hydrogen peroxide at an applied potential of 0.2 V vs Ag/AgCl.

As shown in Figure 6, it was observed that during successive additions of H₂O₂ 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 may be due to the less catalytic activity of Au-NPs for the reduction of H₂O₂⁷⁹.

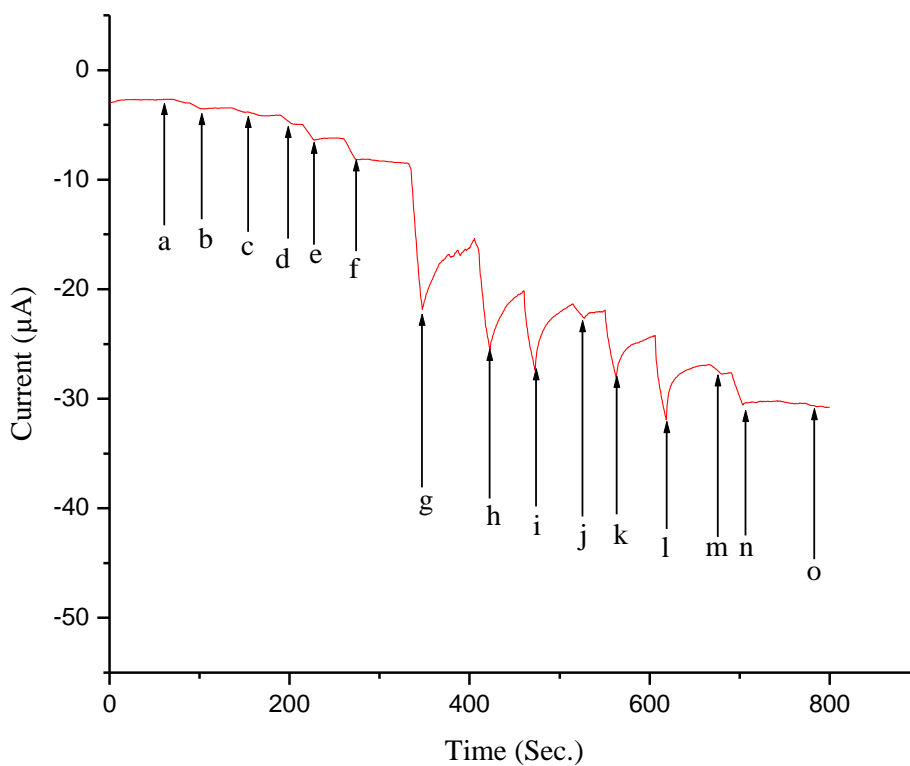


Figure 6: A current-time response curve stirring with successive addition of a) 5.0, b) 10.0, c) 15.0, d) 20.0, e) 30.0, f) 40.0, g) 50.0, h) 60.0, i) 70.0, j) 80.0, k) 90.0, l) 100.0, m) 110.0, n) 120.0 and o) 130.0×10^{-6} mol/L H₂O₂ for glassy carbon electrode modified with Au-NPs in 10.0 mL of 0.1 mol/L pH 7.6 PBS.

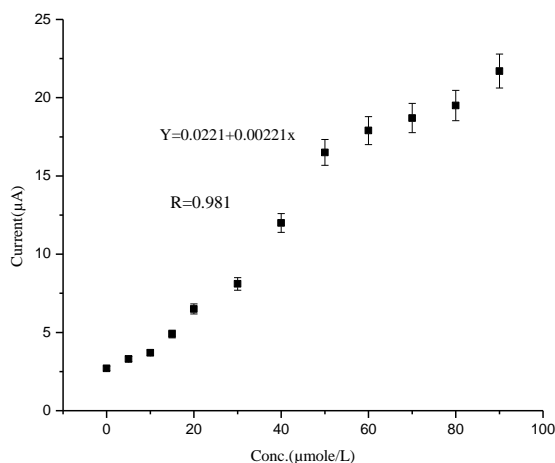


Figure 7: Calibration curve of GCE modified with Au-NPs for the determination of H_2O_2 .

The above calibration curve Figure 7, shows the relationship between the concentration of H_2O_2 and its current response for electrochemical determination of hydrogen peroxide with Au-NPs modified GCE. The value of correlation coefficient ($R = 0.981$) shows the linear relationship between the concentration of H_2O_2 and its current which is not as such appreciable.

5.5. 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 provides some basic information on the characteristic features of Pd reduction and oxidation.

Therefore Figure 8 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 8. The inset linear sweep voltammetry in 1 mol/L H_2SO_4 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 appears due to the oxidation of hydrogen atoms. In the next step the deposited Pd particles are further oxidized to Pd²⁺ 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 to the Pd deposition process on GCE. Based on this observation, reduction potential for potential step deposition of palladium was selected.

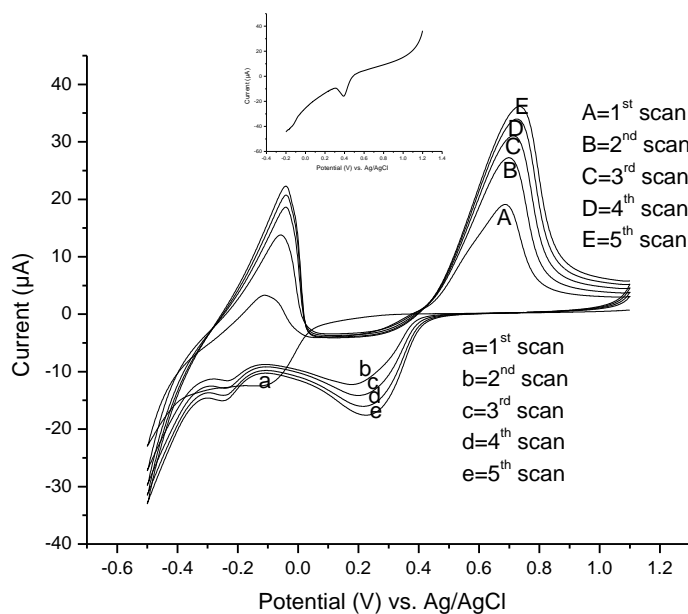


Figure 8: Repeated cyclic voltammogram of Pd electrodeposited on GCE from citrate buffer solution at pH 3 containing PdCl₂ and potential scan between 1.1 and -0.6 V for five cycles scan rate of 0.5 Vs⁻¹.

5.6. Optimization of Experimental Parameters for Pd-NP Modified GCE

5.6.1. Pd-NPs Optimization of Deposition Time

The amount of metal particles on the surface of the electrode has great influence on the performance of the modified electrode. Therefore, the amount of metal particles on the electrode surface depends on the deposition time, which necessitated optimization of the deposition time.

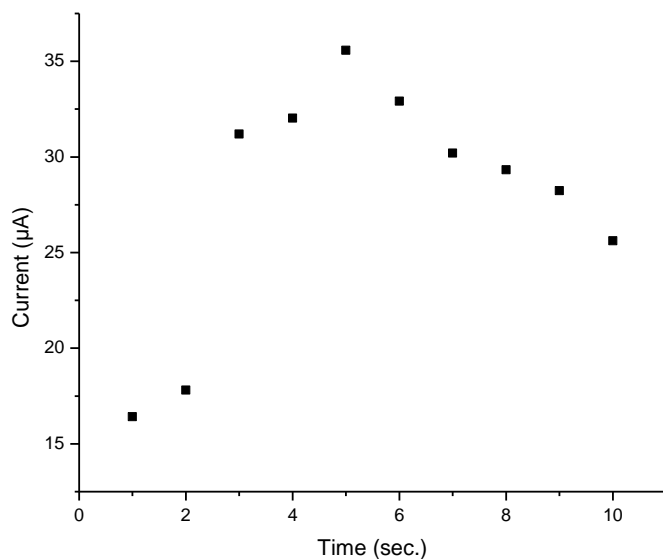


Figure 9: Plot of the response of Pd-NPs modified GCE to various deposition time values containing 0.02 mol/L H_2O_2 . Run at a scan rate of 0.5 Vs^{-1}

The above Figure 9 shows that with increasing of Pd-NPs deposition time for modified GCE, the CV response increases up to 5 second. Further increase of deposition time led to decrease of the response. Therefore, Pd-NPs are deposited on the GCE for 5 second and electrodes prepared under this condition are referred as Pd-NPs modified GC. As we can observed from the result, the current response decreases after 5 second deposition and tells us the electrocatalytic activity decrease as more the electrode surface becomes bulk. Because the amount metal particles deposited depends on its deposition time.

5.6.2. Optimization of pH

In order to improve the performance of the modified electrode, the pH of solution which may influence the response of the electrode was studied. The effect of pH of PBS supporting electrolyte on the electrode response was tested in the range from 5.8 to 8.0, as shown in Figure 9 below.

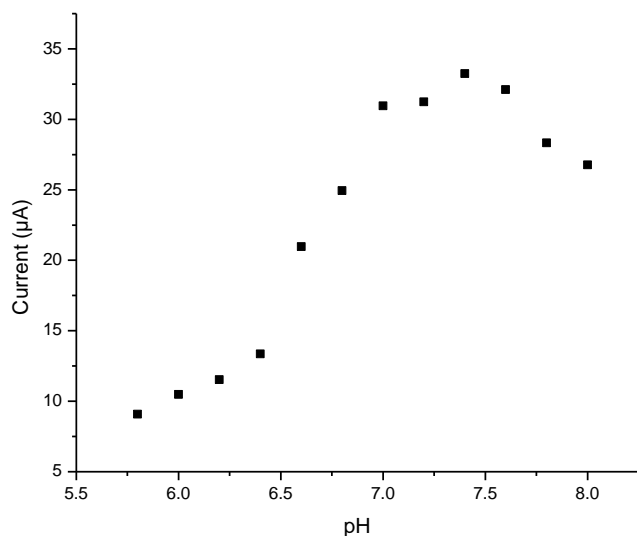
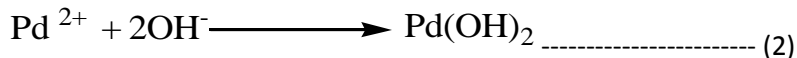


Figure 10: Plot of the response of Pd-NPs modified GCE to various pH values containing 0.02 mol/L H₂O₂. Run at a scan rate of 0.5 Vs⁻¹.

As shown in Figure 9, with increasing of buffer pH, the CV response of the modified electrode increases till pH 7.4. The further increase of buffer pH led to decrease of the response. From the above Figure, pH 7.4 is easily selected for the remainder of the experiments. At lower pH palladium adsorb hydrogen on its surface. It is thought that this possibly forms palladium hydride (PdH₂).



On the other hand at higher pH hydroxide concentration plays a significant role because palladium forms stable of hydroxo complexes.



Therefore, these two possible reasons limit the catalytic activity of palladium at lower and higher pH values.

5.7. Electrochemical Behavior of H_2O_2 at Pd-NPs Modified on GCE

5.7.1. 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 11 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).

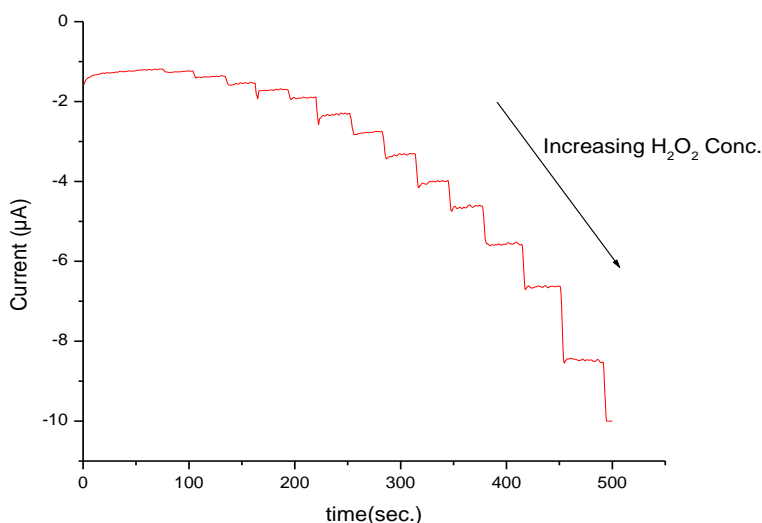


Figure 11: A current-time response curve for successive additions of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0, 130.0, and 200×10^{-6} mol/L H_2O_2 for glassy carbon electrode modified with Pd-NPs in 10.0 mL of 0.1 mol/L, pH 7.4 PBS and stirring with 550 revolutions per second.

As has been shown in Figures 11, a well-defined response was observed during the successive additions of H_2O_2 . The current-time response of the Pd-NPs modified GCE for successive additions of H_2O_2 increases stepwise with each additions of hydrogen peroxide.

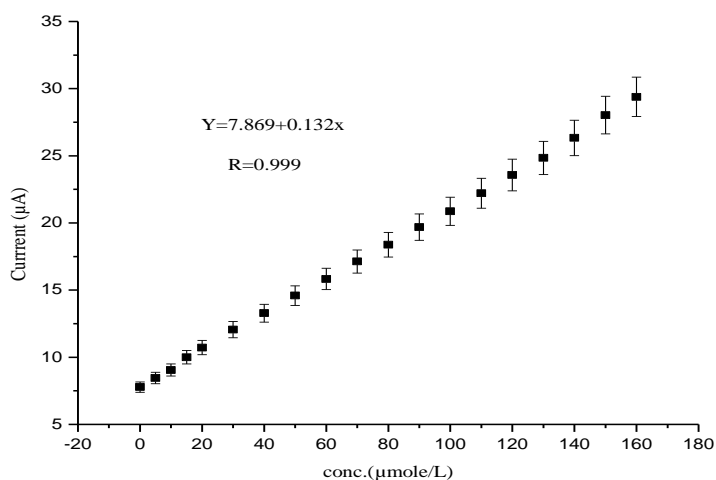


Figure 12: Calibration curve for Pd-NPs modified GCE for determination of H_2O_2 .

The calibration curve shows that the response of Pd-NPs modified GCE to H_2O_2 has a linear relationship in the range of 5×10^{-6} - 200×10^{-6} mol/ L with the regression equation:

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

5.8. Characterization of Pd-Au Co-deposit Modified GCE

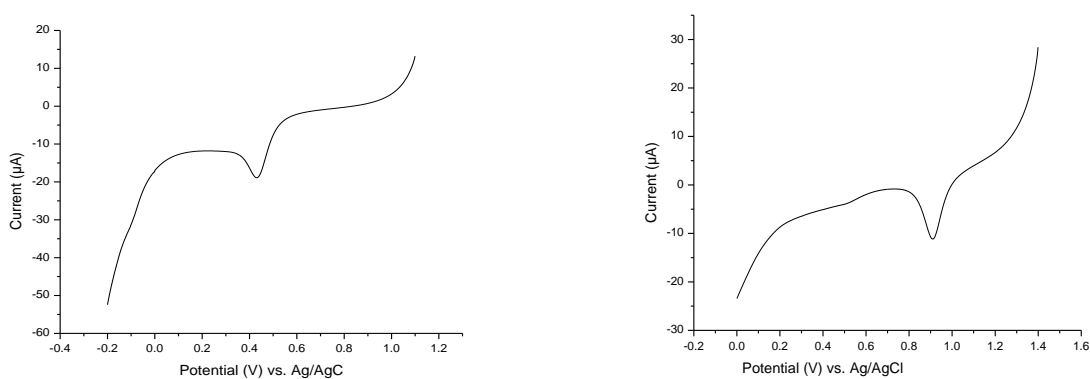


Figure 13: Linear sweep voltammetry of Pd-Au (0.8:0.2) oxide formation followed by reduction for the deposited (0.8:0.2) Pd-Au particles on a GCE surface. A constant potential of 1.1 V is applied for 5 s and scanned in a negative direction at 0.5 Vs^{-1} in 1 mol/L H_2SO_4 . a) deposited Pd from 0.001 mol/L $PdCl_2$ in 0.5 mol/L HNO_3 , and b) deposited Au from 0.001 mol/L $KAuCl_4$ in 0.5 mol/L HNO_3 solution.

Figure 13 (a) shows the linear sweep voltammogram (LSV) of Pd nanoparticles deposition with potential window 1.1 V applied for 5 s and scanned in a negative direction that is -0.2 V and the characteristic current features of Pd reduction around (0.4 V) . Figure 13 (b) shows the linear sweep voltammogram (LSV) of gold nanoparticles deposition with potential window 1.4 V applied for 5 second and scanned in a negative direction that is -0.2 V.

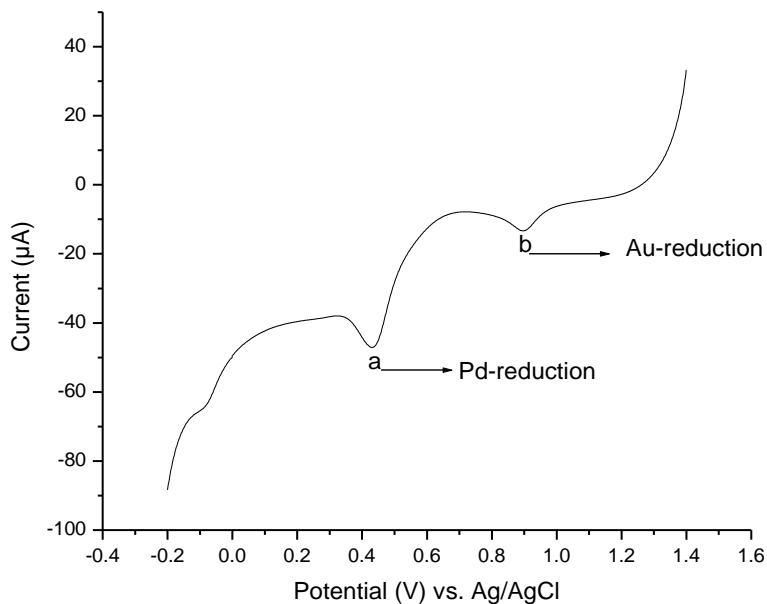


Figure 14: LSV of Pd-Au (0.8:0.2) co-deposit oxide formation followed by reduction for the deposited Pd-Au (0.8:0.2) co-deposit NPs on a GCE. A constant potential of 1.1 V is applied for 5 s and scanned in a negative direction at 0.5 Vs^{-1} in 1 mol/L H_2SO_4 . a) from 0.001 mol/L PdCl_2 in 0.5 mol/L HNO_3 , and b) deposited Au from 0.001 mol/L KAuCl_4 in 0.5 mol/L 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 occurred at +0.40 V and the gold oxide of +0.921 V.

5.8.1. Pd-Au Ratio Comparison

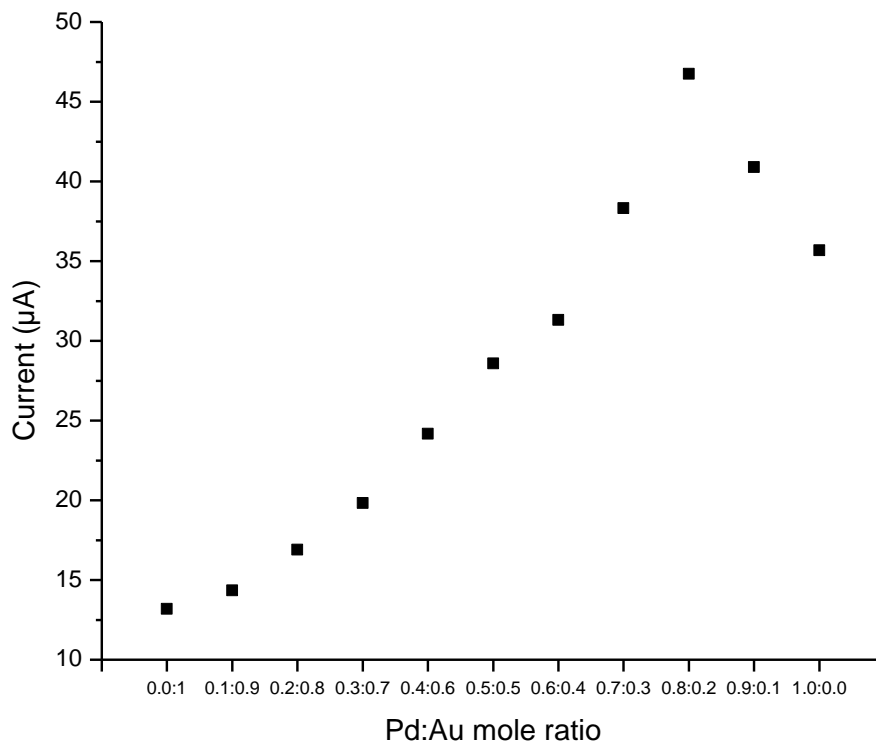


Figure 15: Plot of the response of Pd-Au 1, (0:1) ,2,(0.1:0.9),3, (0.2:0.8),4,(0.3:0.7), 5,(0.4:0.6),6,(0.5:0.5), 7,(0.6:0.4), 8, (0.7:0.3), 9,(0.8:0.2),10, (0.9:0.1), and 11 (1:0) NPs co-deposit modified GCE to pH 7.6 values containing 0.02 mol/L H_2O_2 respectively. Run at a scan rate of 0.5 Vs^{-1} .

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 electroactive 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.

Earlier by other workers⁷⁹, hydrogen peroxide is consumed simultaneously by two alternative reaction pathways, namely, (i) heterogeneous catalytic decomposition and (ii) electrocatalytic reduction. Therefore in this 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 electroreduction 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 process⁸⁰⁻⁸³.

Table 1. Estimation of the area of deposited palladium-gold nanoparticles in 5 second deposition on GCE

Pd-Au mole ratios	Pd surface area (cm²) 1cm² = 424 μc¹⁷	Au surface area (cm²) 1cm² = 400 μc¹⁷
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 H₂SO₄. 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⁻¹ 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⁻¹ for gold oxide formation. Bimetallic catalysts can have considerably more favorable activities, selectivity, and/or deactivation properties than monometallic catalysts,⁸⁴ and the combination of gold and Pd is no exception. Gold is traditionally considered to be a catalytically inert material,⁸⁵ 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 interest⁸⁶⁻⁸⁹.

5.8.2. Amperometric Determination of H₂O₂ Pd-Au (0.8: 0.2)

The amperometric response of the hydrogen peroxide biosensor was investigated by successively adding H₂O₂ to a continuous stirring PBS solution under the optimized conditions. The typical current-time curve of the biosensor is shown in Figure 16. The response current increases with increasing concentration of H₂O₂ as illustrated in Figure 16. In this work, the applied potential of -0.250 V was chosen.

Figure 16 displays the current-time response of the Pd-Au NPs modified GCE with successive injection of hydrogen peroxide at an applied potential of -0.250 V vs the reference electrode (Ag/AgCl).

As shown in the Figure a well-defined response was observed during the successive additions of H₂O₂. The current-time response of the Pd-Au NPs modified GCE for successive additions of H₂O₂ increases stepwise with each additions of hydrogen peroxide.

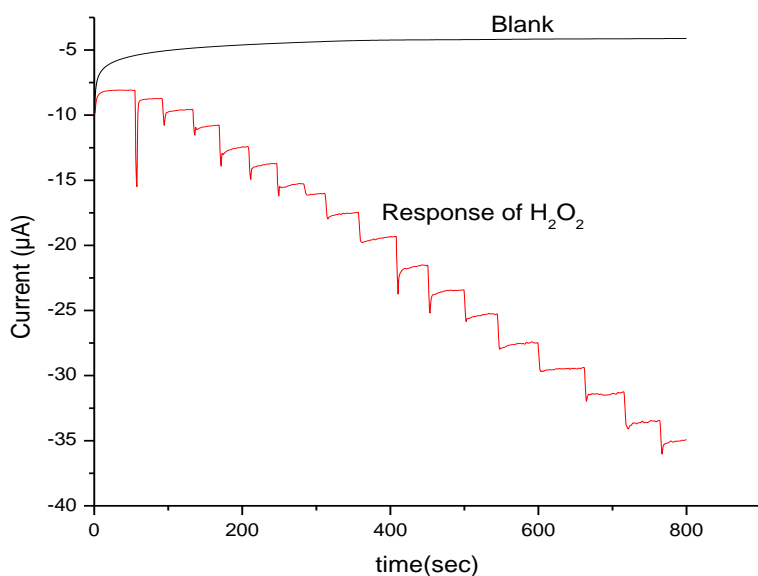


Figure 16: A current-time response curve for successive additions of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0, 130.0, ... and 170.0 $\times 10^{-6}$ mol/L H_2O_2 for glassy carbon electrode modified with Pd-Au (0.8:0.2) NPs in 10.0 mL of 0.1 mol/L of pH 7.6 PBS and with stirring rate of 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 5.0×10^{-6} to 170.0×10^{-6} mol/ L with the regression equation:

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

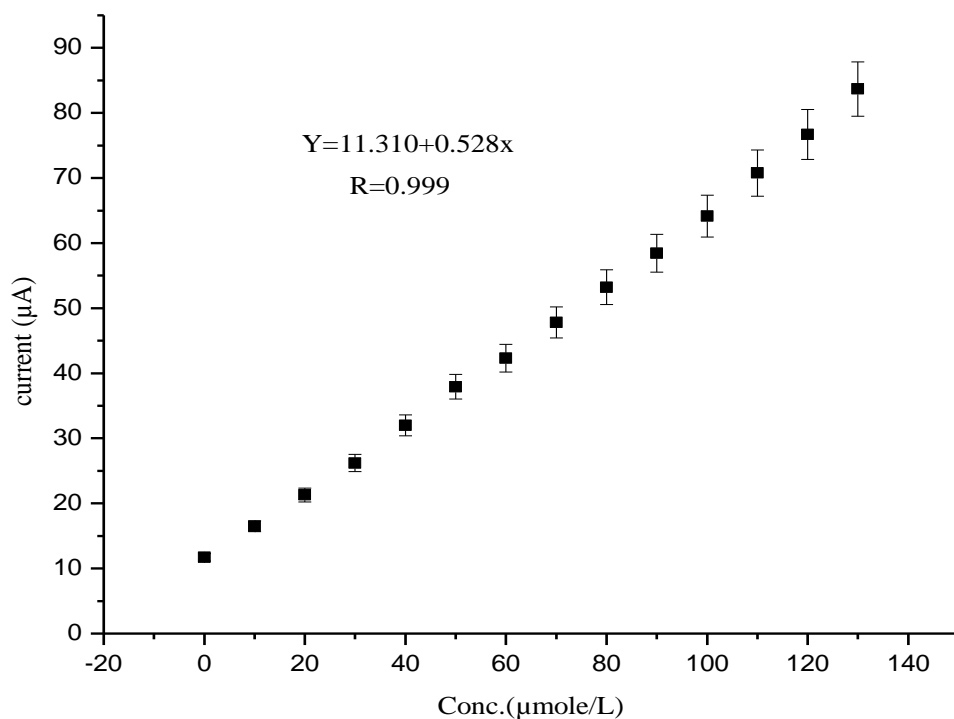


Figure 17: Calibration curve for Pd-Au (0.8:0.2) NPs mole ratio modified GCE for determination of H_2O_2 .

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

5.9. Real Sample Analysis

In order to demonstrate the applicability of the proposed modified electrode for real sample analysis mouth wash was selected to examine this sensor. A sample of mouth wash was bought from a local shop and iodimetric titration was used as a reference method. The concentration of H_2O_2 in the mouth wash was determined by titration was 0.38 ± 0.00015 mol/L. Prior to the determination of H_2O_2 by amperometry, 0.1 mL of mouth wash (the above 0.38 mol/L) was diluted to 100 ML. The sample was carried with three determinations employing the standard additions method set the amperometric measuring potential at - 0.250 V. When the amperometric baseline was moving plainly, the standard H_2O_2 solutions were successively added into the cell

as internal standards. Thus, the concentration of H₂O₂ in real sample can be calculated. The percent error calculated was about 8.21 %.

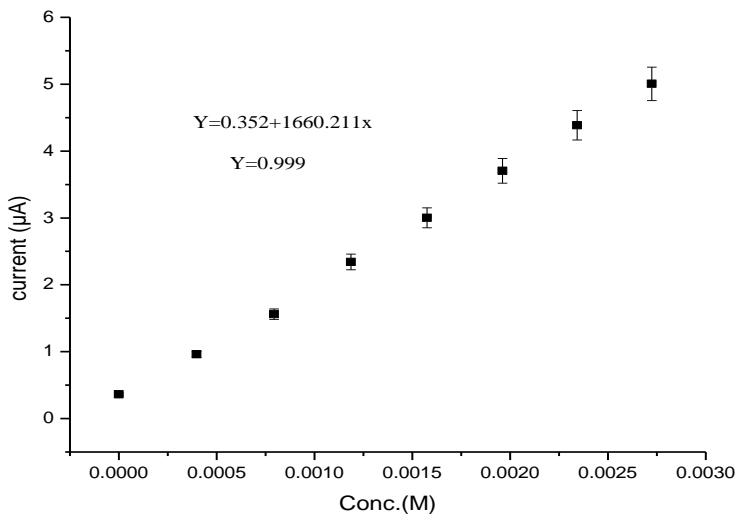


Figure 18: Calibration curve for Pd-Au (0.8:0.2) NPs modified GCE for determination of H₂O₂ from mouth wash.

Table 2. Concentration of H₂O₂ detected in the real sample

Concentration of H ₂ O ₂	Au-NPs modified	P-NPs modified	Pd-Au (0.8:.0.2) modified
Not detected	Not detected	Not detected	0.000414± 0.00011 mol/L
Detected			

From the above Table 2 we can observe the enhanced ability of the mixed Pd-Au (0.8:.0.2) NPs modified GCE ability to detect H₂O₂ from real sample which is difficult to detect with Pd and Au-NPs modified GCE with highly diluted concentration.

5.10. 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 H₂O₂. 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 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 H₂O₂ with the same electrode.

The storage stability of the Pd-Au (0.8:0.2) atomic ratio modified electrode was also investigated by monitoring the response of 0.003 mol/L H₂O₂, 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 reported⁷⁹. 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 H₂O₂.

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 H₂O₂ was tested continuously. During the first 3 days, day by day no change was observed, and after additional 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 2nd week response. This tells us the highly stability of the modified electrode.

5.11. Interference Study for Determination of H₂O₂

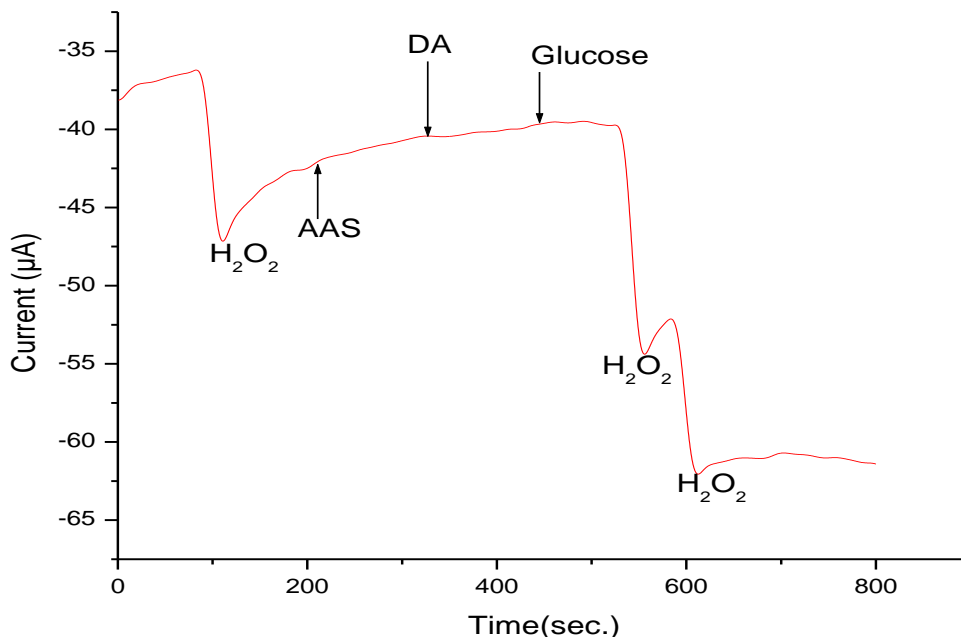


Figure 19: Amperometric response of the hydrogen peroxide upon addition of 0.01mol/L H₂O₂, 0.00015 mol/L ascorbic acid (AA), 0.000005 mol/L dopamine (DA), 0.005 mol/L glucose and 0.01 mol/L H₂O₂ at GCE modified with Pd-Au (0.8:0.2) NPs in 0.1 mol/L PBS and pH 7.6 with stirring rate of 550 revolution per second.

As interference study is important part in the determination H₂O₂ the effect of common interfering electroactive substances such as ascorbic acid (AA), dopamine (DA) and glucose were assessed within the potential window only specific for determination H₂O₂ that is the expected interferences never show reduction current response with the selected potential and above Figure 19 shows that the amperometric response for the injection of H₂O₂ was free from the expected interference such as AAS, DA, and glucose. From this information the current response is due to reduction of H₂O₂ and this due to the specificity of potential window for H₂O₂ and exclusively of the sensor for H₂O₂.

6. Conclusions

In this study, the electrocatalytic reduction of hydrogen peroxide on a glassy carbon (GC) 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 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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Appendices

Au-NPs Optimization of pH For Determination of Hydrogen Peroxide

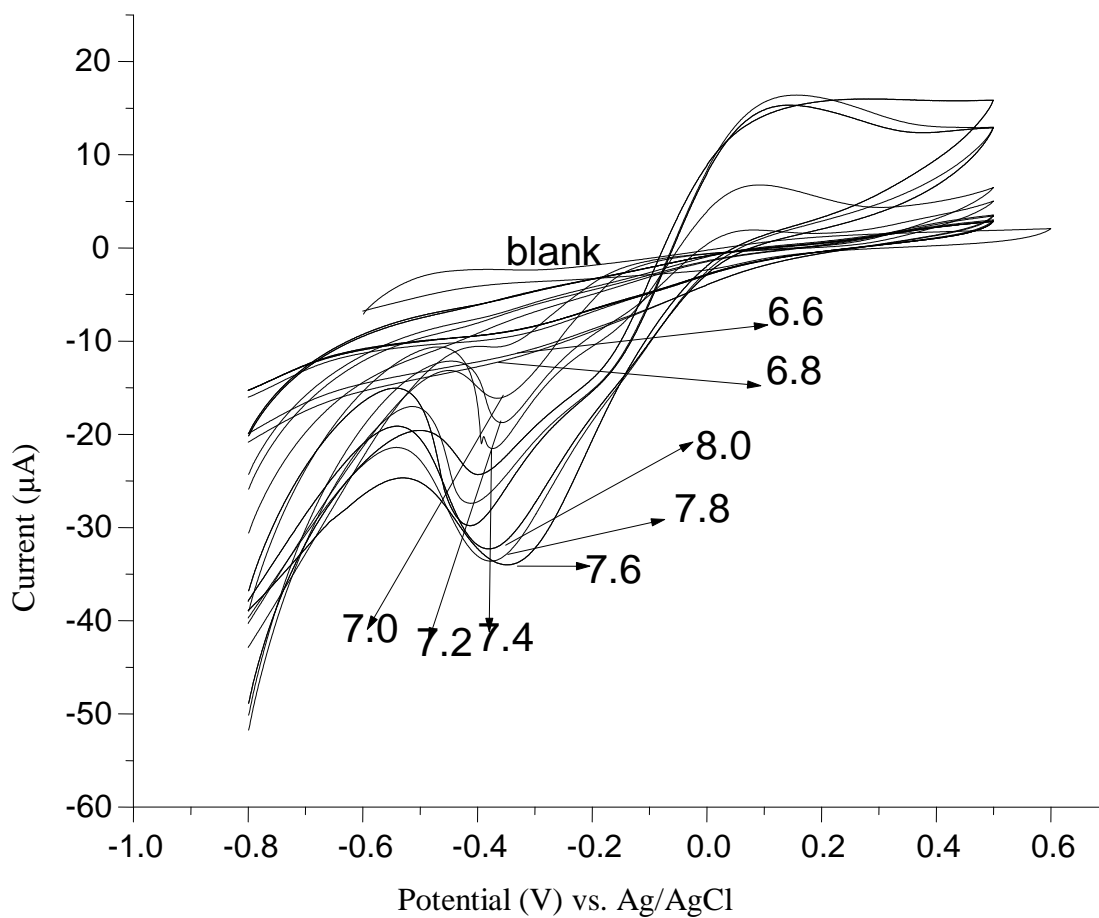


Figure A: Cyclic voltammogram detailing the response of Au-NPs modified GCE to various pH values containing 0.04 mol/L H_2O_2 . Run at a scan rate of $0.5 V^{-1}$.

Au-NPs Optimization of Deposition Time For Determination of H₂O₂

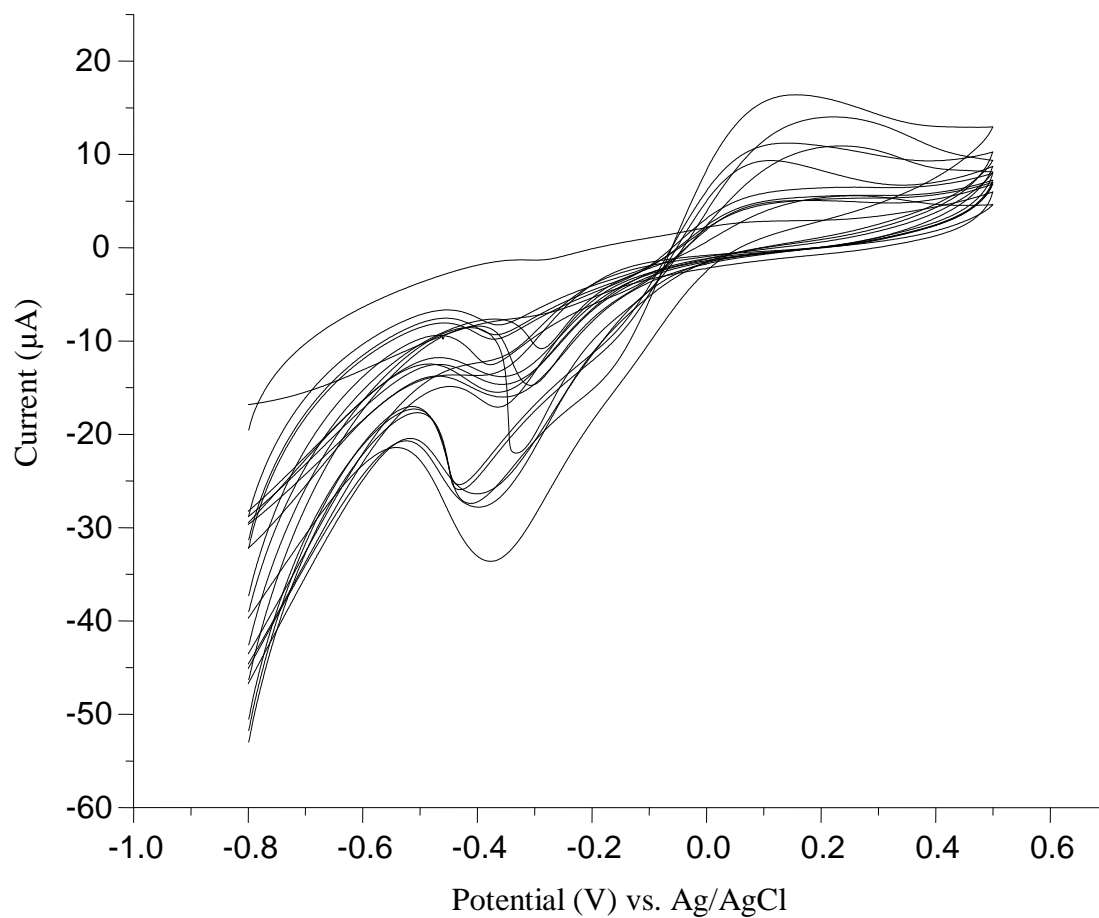


Figure B: Cyclic voltammogram detailing the influence of deposition time for the response of Au nanoparticles modified GCE to 0.04 mol/L H₂O₂. Run at a scan rate of 50 mVs⁻¹ in PBS (0.1 mol/L, pH 7.6).

Pd-Nps Optimization of Deposition Time For Determination Of H₂O₂.

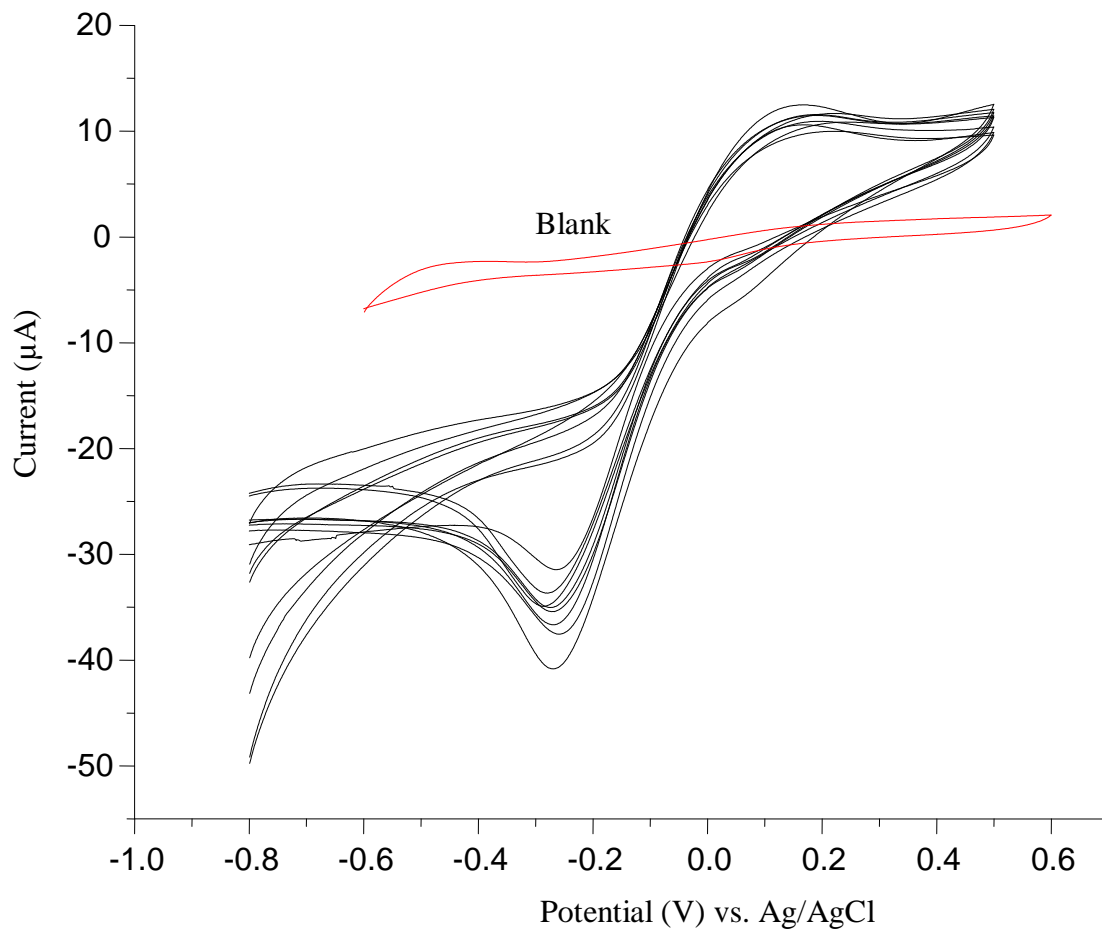


Figure C: Cyclic voltammogram detailing the influence of deposition time for the response of Pd nanoparticles modified GCE to 0.02 mol/L H₂O₂. Run at a scan rate of 50 mVs⁻¹ in PBS (0.1 mol/L, pH 7.4).

CV of Pd-Au Ratio Comparison

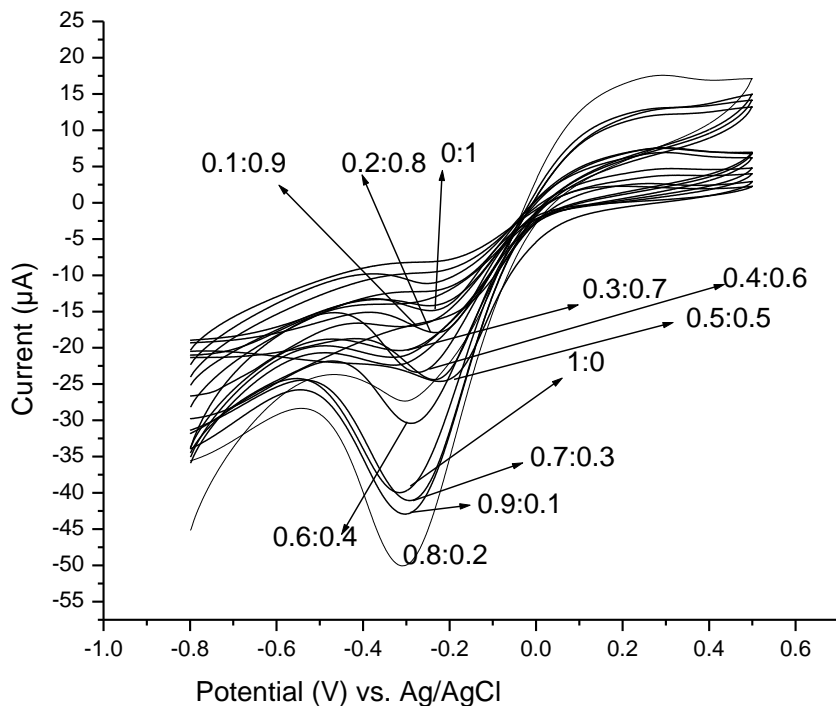


Figure D: Cyclic voltammogram to the response of Pd-Au 1, (0:1), 2, (0.1:0.9), 3, (0.2:0.8), 4, (0.3:0.7), 5, (0.4:0.6), 6, (0.5:0.5), 7, (0.6:0.4), 8, (1:0), 9, (0.7:0.3), 10, (0.9:0.1), and 11 (0.8:0.2) NPs co-deposit modified GCE to pH 7.6 values containing 0.02 mol/L H₂O₂ respectively. Run at a scan rate of 0.5 Vs⁻¹.