Original Article

Fabrication of Advanced Cu/Self Assembled Monolayer/ Prussian blue Electrode as Electrochemical Sensor for H_2O_2 Detection

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Abstract

Background: Electrochemical analyses are now very commonly used in many analytical applications.

Objectives: Here, the copper electrode is modified by coating Prussian Blue (PB) with the help of self-assembled monolayer (SAM) as an intermediate layer.

Materials and Methods: The confirmation of SAM and PB coating over the copper electrode was confirmed using water contact angle analysis, ellipsometry study, electrochemical impedance studies, etc. The sensitivity of Cu/SAM/PB electrode to hydrogen peroxide was studied using amperometry measurements.

Results: Here, the SAM precursor taken was 1-Dodecanethiol ($C_{12}H_{26}SH$) for binding the PB nanoparticles to the copper electrode. The characterization of PB nanoparticles had been carried out by Raman spectroscopy, UV-Vis spectroscopy.

Conclusions: The modified electrode was able to detect hydrogen peroxide even in 10 μM concentrations, whereas for bare copper the detection starts only at 10 mM.

Key words : Prussian blue, hydrogen peroxide, self-assembled monolayer, electrochemical sensor, amperometry.

Introduction

Electrochemistry in the field of nano technology plays a significant role in fabricating various sensors used for detection of molecules at very low concentrations. The electrochemical sensor's performance can be improved by the introduction of nano structural properties to the sensor. Sensors play a vital role for monitoring environmental factors such as temperature, weather conditions, earth, sea transportation, chemical pollutants and biological substances.[1] Hydrogen peroxide is present in many biological reactions as the main product of several oxidase reactions. Here, the detection phenomenon was mainly due to the presence of Prussian Blue ($Fe_4[Fe(CN)_6]_3$) (PB) which has perfect catalytic activity towards some low molecular weight molecules (such as O_2, H_2O_2, hydrazine) due to its unique zeolite structure. PB incorporated electrochemical sensors have been widely investigated for the H_2O_2 detection. It was

reported that at optimized conditions, the calculated bimolecular rate constant for the reduction of H_2O_2 was 3×10^3 M^{-1} s^{-1}, which was very similar to the measured values for the peroxidase enzyme (2×10^4 M^{-1} s^{-1}).[2] Electrochemical properties such as formal potential, sensitivity, stability and electron transfer rate constants of the PB and its corresponding redox state depend on deposition method, pH, molecular structure, concentration of the supporting electrolyte, etc.[3] Therefore, sensor properties can be tuned by varying those parameters. Leili Aghebati-Maleki et al, designed and fabricated H_2O_2 biosensor using magnesium oxide nano particles.[4] These sensors detect H_2O_2 with very low concentration between 50-190 μM. Novel carbon nanoparticles are used to prepare electrochemical sensors. The graphene precursor, Graphene Oxide (GO), was selfassembled on Glassy Carbon Electrode (GCE) through a diamine linker which was preassembled on GCE by electrostatic interaction between the positively

charged amine and the negatively charged layers of graphene oxide (GO).[5] The alkane thiols form Self-Assembled Monolayers (SAM) on the iron-based electrodes. The hydrogen evolution by the alkanethiols was drastically suppressed and the degree of suppression was found to be greater than 90%, and the effectiveness of the alkanethiol increased with the increase in chain length.[6-8] The self-assembling of n-dodecanethiol, n-dodecane selenol, didodecyldisulfide and didodecyldise-lenide on copper substrate was studied in real time by in situ electrochemical impedance spectroscopy was studied by Thangamuthu Madasamy et al.[9] Regina Mazˇeikiene et alstudied the performance of PBNCs/rGO nanocomposites as amperometric sensor toward reduction of H_2O_2 and also studied the electrochemical behavior of graphene sheets attached to a SAM on a gold electrode.[10] SAM of thiols on gold electrodes can be prepared by gold electrochemical deposition on platinum wire. The experimental results of Xiang Xie et al have shown that the analytical performances of these electrodes were comparable with those obtained in the case of manufactured gold electrodes.[11] Electrochemical Impedance Spectroscopy (EIS) was used to analyze the different chain lengths of SAM of alkanethiols on gold electrode.[12] The electron-transfer resistance of the alkyl chain increases with increase in chain length of the SAM layer. The formation of a self-assembled chemisorbed layer of urease on gold for urea sensor had been studied by X-ray photo- electron spectroscopy (XPS) and their electrochemical properties are characterized by the amperometry method.[13,14] Overall, the chemistry between the PB and the hydrogen peroxide were good, but there needs a different model or a prototype to get a high sensitivity as well as to get a commercial product for sensing.

Materials and Methodology

The schematic representation of experimental procedure for the preparation of modified Cu/SAM/PB electrode for H2O2 sensing is shown in Figure 1
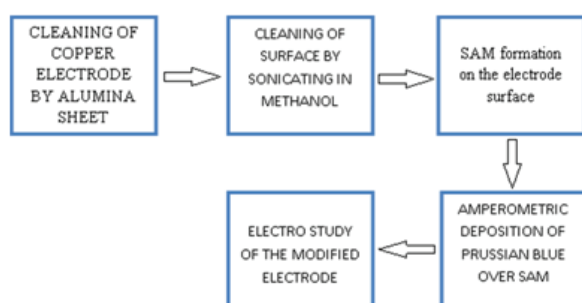

Figure 1: Schematic representation of the materials and methodology

Preparation of Prussian Blue (PB) nano powder

The PB nanoparticles were prepared separately for the characterization purpose. Here 0.1 mM of Iron chloride ($FeCl_3$) and equimolar of potassium ferricy-anide $K_4[Fe(CN)_6]_3^-$ were taken and dissolved sepa-rately. After the iron chloride solution was added drop wise into potassium ferricyanide. The final product forms a deep blue color which confirms the formation of Prussian blue. This was being confirmed through ultraviolet spectroscopy, Raman Scattering and by Scanning electron microscope.

Chemical Background for electrode

Copper Electrode:

The copper electrode works on the principle of redox reaction in the presence of the metal here in the sense copper. Copper electrodes are most widely used to test cathodic corrosion protection control systems. The equation that follows the sensing characteristics can be presented as follows,

$$Cu^{2+} + 2e- \rightarrow Cu \text{ (metal)} \qquad (1)$$
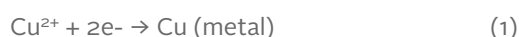
The reaction shows fast electrode kinetics and reversible characteristics indicating larger current can be passed through the electrode with redox reaction of 100% efficiency. The potential of a copper electrode is +0.314 volts with respect to the standard hydrogen electrode.

1-Dodecane thiol ($C_{12}H_{26}SH$):

1-Dodecanethiol is a long branched organic molecule having a head and a tail part. These molecules have a highly hydrophobic $C_{12}H_{26}SH$ tail group which is pointing outwards and forms a more hydrophobic surface. In the case of alkanethiols on copper surface, they are strongly chemisorbed on the copper surface by the formation of a covalent-like bond between copper and sulphur atoms following cleavage of a sulphur-hydrogen bond. Densely packed SAM layer on the surface were achieved by the chemisorption of alkanethiols.

Cleaning of Electrode Surface

Cleaning of the Cu electrode surface prior to fabrication of the biosensor was necessary for the formation of the SAM layer. Piranha, an oxidant solution was used to remove the organic contami-nants on the surface of the electrode. The piranha solution was prepared by mixing 30% hydrogen peroxide and concentrated sulphuric acid in the ratio 1:3. The electrode was rinsed in piranha solution for 2 minutes and then washed with bi-distilled water. 0.05 μm alumina slurry was used to polish the electrode and again washed with bi-distilled water.

To remove the slurry particles on the electrode surface, the electrode was subjected to ultrasound bath for 5 minutess and then cleaned with methanol. All these steps were performed to avoid accumulation of redox reaction products which causes uneven coating of SAM layer.

Monolayer Formation over the Surface of Copper:

After a careful cleaning of the electrode, the electrode was immersed in the dodecanethiol (1mM) in methanol solution for different time periods ranging from (1h, 2h and 3h) to find out the full surface coverage of dodecanethiol over the copper surface. The surface coverage was found out electrochemically using EIS.

Deposition of Prussian Blue (PB) Nanoparticle Over the Monolayer

Prussian Blue (PB) was amperometrically deposited on the Cu/SAM electrode with the precursor solution containing 2 mM in $FeCl_3$, 2 mM in $K_3[Fe(CN)_6]$, 0.1 M in KCl and 0.1 M in HCl with a working potential of +400 mV applied for 30 s. The electrochemical sensing behavior was carried out using the prepared PB/SAM/Cu electrode.

Results and Discussion

Characterization for Prussian Blue Nanoparticles

SEM Analysis:

The SEM analysis was carried out using Hitachi SUI510, Scanning Electron Microscope (SEM). The SEM image shows the morphology of the PB nanoparticles. From Figure 2 the morphology of PB shows a cube like structures with uniform arrangement. From the SEM analysis the size of the PB nanoparticle ranges from 150 to 200 nm.
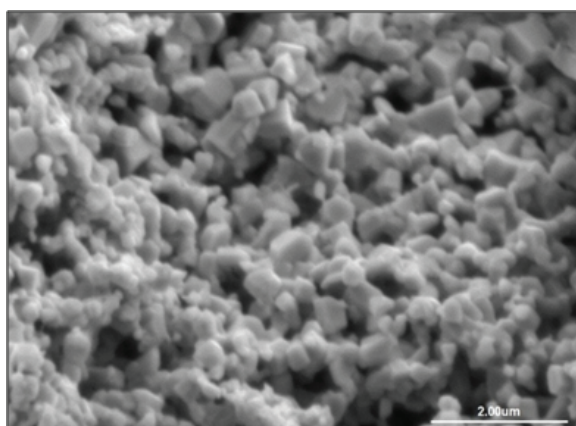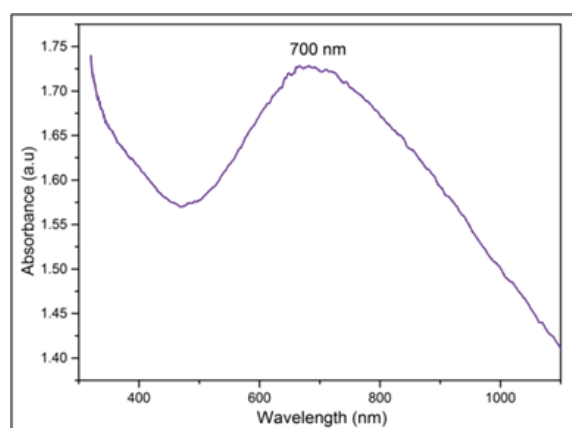

Figure 3: UV-Vis spectrum of PB nanoparticles

U-V Spectroscopic Analysis:

Figure 3 shows the UV-Vis spectra of PB nanoparticles analyzed in the range from 300 to 1100 nm using Carry 5000 Scan, Varion. The UV-Vis spectra showed a broad peak at lower energy wavelength of the visible region (~700 nm). The peak at the weaker band is due to the intense charge transfer absorption band of polymeric sequence Fe^{2+}-CN-Fe^{3+} found in PB.[15] This peak is the charact-eristic peak for the PB nanoparticles. Also, absorption was found towards the higher wavelength region (~300 nm).

Raman Spectroscopy

The Raman spectroscopy of PB nanoparticles was taken using RenishawInvia Raman Microscope, U.K. The source He-Ne laser 633 nm, 18 mW was employed to obtain the Raman spectrum. From Figure 4, the Raman spectra of PB show a characteristic peak at 2154 cm^{-1} which was due to the stretching vibration of carbon-nitrogen triple bond group present in the molecule.[16] The remaining peaks at 278.4 cm^{-1} and 532.29 cm^{-1} were originated due to the presence of co-precipitated ferricyanide ion, indicating the formation of PB nano particles.
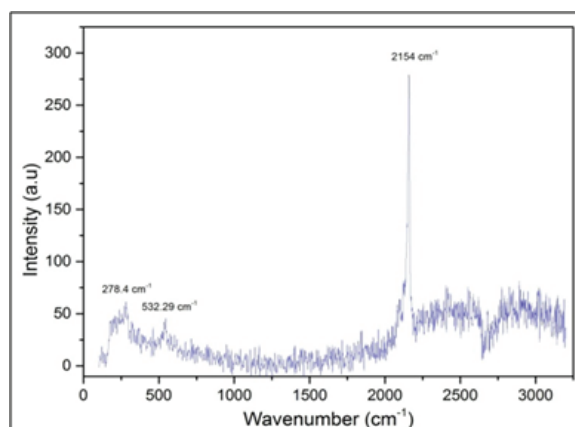
Figure 2: SEM micrograph of the Prussian Blue nanoparticles

Figure 4: Raman spectrum for Prussian Blue nanoparticles

Original Article

Fabrication of Advanced Cu/Self Assembled Monolayer/Prussian blue Electrode as Electrochemical Sensor for H_2O_2 Detection
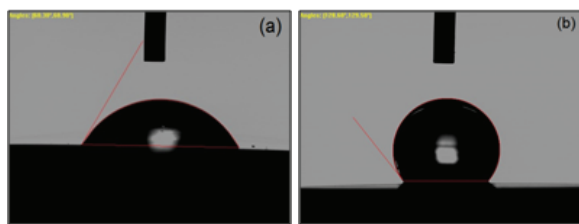
Volume 9, Number 2

Figure 5: Contact angle analysis for (a) copper electrode before modification (b) copper electrode after modification

Confirmation studies for the formation of SAM layer

Contact Angle Analysis:

The contact angle analysis was done to determine the hydrophobic property of the copper surface before and after modifying with the dodecanethiol. It is to be noted that the entire hydrophilic surface like copper and the other metals have an angle below 90°, while the hydrophobic surface has an angle above 90°. The above Figure 5 shows the contact angle for a bare copper and for the modified surface as 60.30° and 128.60° respectively. From the Figure 5 we can confirm the formation of monolayer over the copper surface as the contact angle increases drastically to 128.60° as the SAM layer has hydrophobic tails.
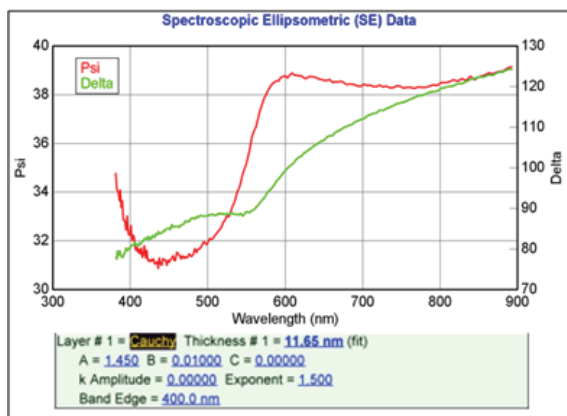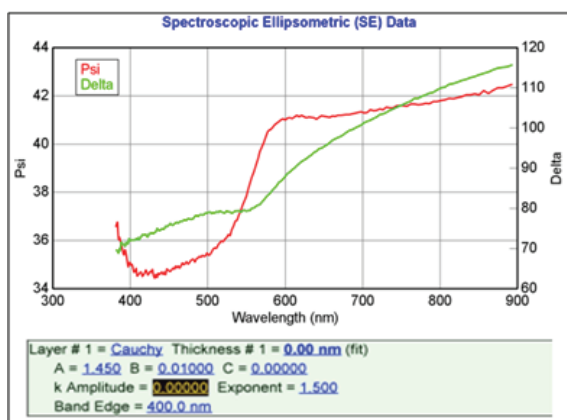

Ellipsometric Analysis:

Ellipsometric analysis was carried out to find the thickness of the SAM layer formed on the surface of the copper electrode. By knowing the thickness of the SAM layer, we can predict the number of SAM layer (s) formed over the copper substrate.

The spectra results show that the formed monolayer had a thickness of 11.65 nm with respect to the copper surface was shown in Fig. 6 (a – b). Also, it confirms that the formed layer is a monolayer since the total chain length of the dodecanethiol is approximately 12 nm. Therefore, there was no multilayer formation of SAM over the copper electrode.

Electrochemical Studies:

The whole electrochemical study was carried out using a three-electrode system. The impedance analysis for bare and a modified copper electrode have been carried out in 0.1 M solution of NaCl (electrolyte) to confirm that the copper surface was fully covered with the monolayer without any defect.

From Figure 7 the modified electrode shows a high charge transfer resistance when compared to the bare copper electrode. The surface coverage of monolayer over the electrode can be calculated by the formula, $1-\theta = Rct\bullet / Rct$;[2] where, $Rct\bullet$ and Rct are the charge transfer at bare electrode and the charge transfer at modified electrode respectively. The surface coverage of monolayer over the bare electrode was only 42% when immersed in a time period of 1 h. So, the monolayer formation time was increased up to 3 h to optimize the time essential for SAM layer formation with almost 100% surface coverage for better results.
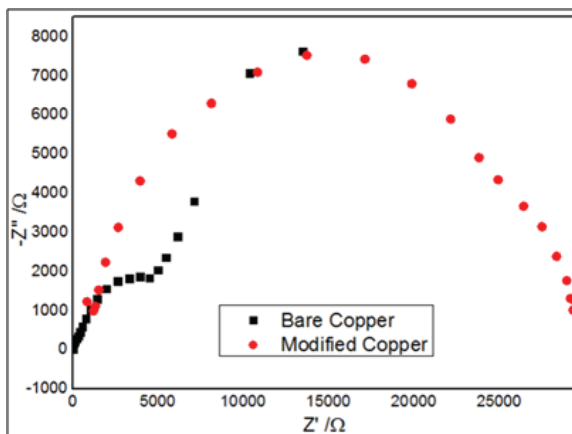

Figure 6: Ellipsometry analyses for the (a) bare electrode (b) modified electrode

Figure 7: Impedance spectra for the bare and modified electrode in 0.1M of NaCl solution

Time Period	Surface Coverage,θ
At 1 Hour modification	Θ = 42%
At 2 Hour modification	Θ = 83.9%
At 3 Hour Modification	Θ = 98.23%

Table 1: Percentage of Surface coverage of the dodecanethiol over copper electrode with different time intervals

Table 1 shows the surface coverage of SAM over the bare electrode for 1, 2, and 3 hours. At 3 h almost 98.23% surface area of copper electrode was coated with SAM layer.

Amperometric Sensor

Amperometric method was used to sense the presence of H2O2 using modified electrode Cu/SAM/PB. Amperometric sensor studies were carried out in an electrolyte solution containing Phosphate buffer with pH 6.7 at an applied voltage of -0.4 V. To the above electrolyte, stepwise addition of stock solution (H_2O_2) was carried out from μM to mM concentration. At first the amperometric detection was performed for the bare copper electrode to study the sensing capacity over hydrogen peroxide. The typical current-time response for bare copper

Figure 8: : Amperometric detection of hydrogen peroxide for (a) bare electrode and (b) modified Cu/SAM/PB electrode

with successive addition of hydrogen peroxide was shown in Figure 8 (a). The amperometric measurement was taken with different time interval with a different concentration range of stock solution. The concentration of stock solutions was gradually increased up to a steady increase of current. The results show that the bare copper started sensing H2O2 only at 10 mM concentration.

Figure 8 (b) shows the current-time plot for the modified electrode Cu/SAM/PB with the addition H_2O_2. The concentration of H_2O_2 was added step wise with an initial addition of 5 μM. The modified copper electrode senses the presence of H2O2 at a concentration of 10 μM. This indicates that the prepared modified electrode can detect H2O2 at a concentration 1000 times lesser concentration than that of the detection limit of bare copper. The efficient sensing of Cu/SAM/PB modified electrode is due to the nano level assembly of 1-Dodecane thiol over the copper electrode which gives more sensitivity than the bare surface. This is due to the more active sites present in the SAM layer than on the bare surface. Also, the surface to volume ratio plays a major role in the efficient sensing capability of the Cu/SAM/PB electrode. The reproducibility of the fabricated electrode was checked by repeating the experiment for three times and the difference in change in response was negligible.
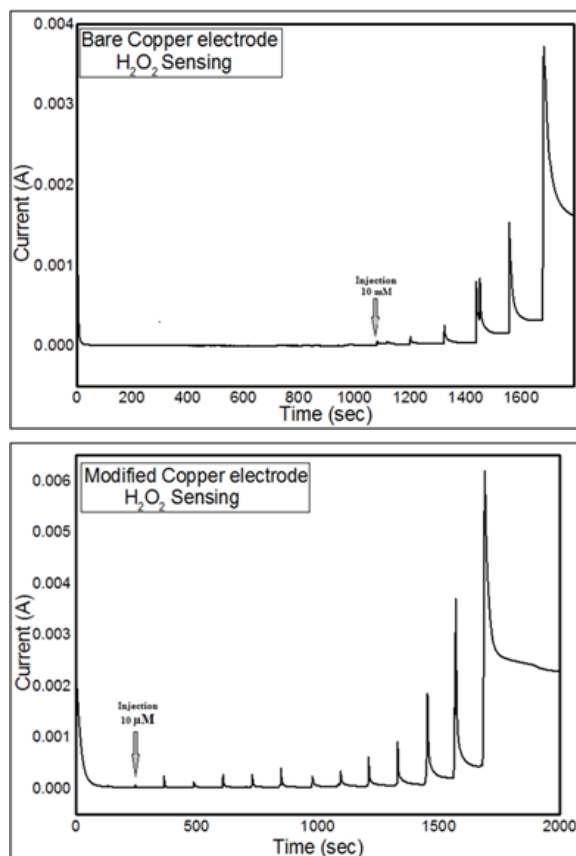
The common interfering species such as ascorbic acid or uric acid were added to the electrolyte solution to study the effect of such species in the sensing capability of the fabricated electrode. The sensitivity of modified electrode starts with addition of 10 μM, where the increment of current was noticed. With the introduction of same of quantity of interfering species there was no considerable change in sensitivity, indicating the stability of the sensor.

Conclusion

In summary, the electrode was being successfully modified by depositing the 1-Dodecanethiol over the clean copper surface. After the formation of monolayer over the electrode surface, Prussian Blue was amperometrically deposited by means of chronoamperometric method. Finally, the modified electrode looks like a sandwich model as Cu/SAM/PB. The Prussian blue was characterized using UV-Vis spectra, Raman spectroscopy and by SEM analysis. The monolayer modified electrode was confirmed using the contact angle, ellipsometry and electrochemical impedance. The modified electrode (Cu/SAM/PB) was studied for sensor application using amperometric method for the detection of hydrogen peroxide. The modified electrode has the capacity to sense the hydrogen peroxide even at 10 μM concentrations while the bare electrode senses only millimolar concentra

Original Article
Fabrication of Advanced Cu/Self Assembled Monolayer/Prussian blue
Electrode as Electrochemical Sensor for H$_2$O$_2$ Detection
Volume 9, Number 2

tions. Future work involves fabricating sensors with different nanostructures to detect nanomolar concentrations of H$_2$O$_2$.

Acknowledgments

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