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Carbon paste electrode with covalently immobilized thionine for electrochemical sensing of hydrogen peroxide

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Abstract. A water-soluble redox mediator, thionin was covalently immobilized to the functionalized graphite powder and a carbon paste electrode was fabricated from this modified graphite powder. The immobilization procedure proved to be effective in anchoring the thionin mediator in the graphite electrode setup without any leakage problem during the electrochemical studies. The covalent immobilization of the thionin mediator was studied with FT-IR and the electrochemical response of the thionin carbon paste electrode was optimized on varying the supporting electrolyte, pH and scan rate. The modified electrode exhibited well-defined electrocatalytic activity towards the reduction of H₂O₂ at a lower potential of -0.266 V with good sensitivity. The developed amperometric sensor was efficient towards H₂O₂ in the linear range from 2.46 x 10⁻⁵ M to 4.76 x 10⁻³ M, with a detection limit of 1.47 x 10⁻⁵ M respectively. Important advantages of this sensor are its excellent electrochemical performance, simple fabrication, easy renewability, reproducible analytical results, acceptable accuracy and good operational and long-term stability.

1. Introduction

Modification of electrode surface has been an active area of research due to their application in sensor, electrosynthesis, electrocatalysis and selective electrodes for specificity and selectivity [1-4]. Among the various electrode materials, carbon based electrodes are finding an important place in material science, electrochemistry and biotechnological application [5-7] due to distinct advantages of very small background currents, chemically inert, broad potential window, inexpensive and aptness for various sensing applications. These attractive features are the reason for the development of various carbon based electroanalytical sensors. Tailor make electrodes by the derivatisation of carbon surfaces has offered distinct merits towards catalysis and biological applications attracting substantial interest in the recent years [8,9]. Carbon paste electrodes (CPEs), the popular carbon electrodes are prepared by mixing an electrically conducting graphite powder and a non-conducting liquid paste. CPEs have been extensively used as a substrate for the bulk modification of electrodes due to the attractive features of composite in nature, easy modification, renewability and miniaturization [10,11]. Determination of hydrogen peroxide has been an important area of research since the last century, due to its significance in the domains of pharmaceutical, biological, chemical, plastic, food processing industries and environmental studies [12-15]. Also, H₂O₂ is enzymatically generated or consumed by a large number of oxidase enzymes, which forms the basis for the construction of numerous biosensors



[16,17]. Hence selective and sensitive determination of H_2O_2 with reliable, rapid and economical method becomes always an important task.

Many methods based on various principles have been developed for the estimation of H_2O_2 including titrimetry [18], spectrophotometry [19], fluorimetry [20], fiber-optic [21], chemiluminescence [22]. However, these methods suffer from complicated, time consuming procedures, subject to interference and demand expensive reagents. In order to circumvent these problems, a large number of efforts have been made to design simple and effective method for the determination of H_2O_2 . Besides all the techniques mentioned above, electrochemical methods [23-25] have proven to be promising for this purpose since they are simple, speedy and reach lower detection limit offering quick response times based on direct oxidation or reduction reactions with H_2O_2 . Continuous efforts have been made towards construction of chemical sensors for the analysis of biologically, medicinally and environmentally significant analyte using various immobilization strategies [26-29].

Chemically modified CPEs are prepared traditionally by mixing the mediator, graphite and pasting liquid. A number of water soluble redox mediators are available which could not be employed as mediators since they may not adhere to the surface when in contact with aqueous solution. A right immobilization strategy to anchor the mediator onto electrode surface is crucial in order to realize high sensitivity, performance stability and long term storage stability of a sensor. Hence an attempt has been made in this work to covalently immobilize a redox mediator with graphite powder, so that a water soluble redox mediator can be used for electrode preparation. The mediator chosen here is thionin (TH), a water soluble phenothiazine organic dye containing two primary amino groups. The mediator is immobilized to graphite powder using the amino group of thionin, to achieve a bulk modified graphite that can be used for carbon paste electrode preparation. This will render the effective use of thionin as a redox mediator without any associated leaching problem. Functionalisation of graphite powder was carried out prior to covalent immobilization of the thionin mediator. Surface renewable carbon paste electrode developed from thionin immobilized graphite powder has satisfactory physical and chemical stability to retain the mediator performance for electroanalysis. H_2O_2 determination has been chosen as an example to exemplify the utility of the modified electrode.

2. Experimental

2.1. Reagents and solutions

Thionin was procured from Himedia. Graphite powder (1-2 μ) was procured from Aldrich. Hydrogen peroxide (50 %) received from Merck was diluted freshly before electrochemical studies. All other chemicals employed were of analytical grade. The electrolyte pH was adjusted using KH_2PO_4 , K_2HPO_4 , H_3PO_4 and NaOH. Double distilled water was utilised for dilutions and preparation of solution.

2.2. Apparatus

Electrochemical studies were performed with CHI 400 A Electrochemical Analyzer with conventional three electrode system. Thionin immobilized carbon paste electrode (TH-CPE) served as indicator electrode and a platinum wire was employed as counter electrode. All potentials are referenced to saturated calomel electrode (SCE). Convective transfer for the amperometric measurements were given with magnetic stirrer. pH measurements were done with Elico LI 120 pH meter. Anaerobic conditions were obtained by saturating the solutions with oxygen free nitrogen for 15 min. before commencing the electrochemical studies.

2.3. Immobilization strategy of thionin to graphite

Prior to the covalent immobilization of thionin, graphite powder was first activated to functionalize with carboxyl functional groups. Graphite powder was refluxed with 5 M HNO_3 for 7 h. Thorough washing of the oxidised graphite powder was carried out to bring the pH to 7.00 followed by heating

in an oven at 150° C for 13 h [30]. Thionyl chloride was utilized for successive conversion of carboxylic groups to acid chloride. Oxidised graphite was refluxed for 5 h with 5% SOCl₂ in toluene. Excess thionyl chloride was removed by generous washing with toluene and dried in an oven at 150° C for 13 h [30]. Covalent immobilization of thionin was performed by refluxing 30 mg of thionin with 2 g of functionalized graphite powder in DMF for 12 h to make the primary amino groups on the thionine organic dye to react with the acid chloride on the graphite powder to result in an amide linkage. Unreacted dye was removed by washing subsequently with DMF and isopropanol and finally dried at room temperature for 4 h.

2.4. Preparation of thionin modified CPE (TH-CPE)

The thionin carbon paste electrode (TH-CPE) was fabricated using thionin immobilized graphite powder and silicone oil. A homogeneous paste was made by blending thoroughly 30 mg of thionin immobilized graphite powder with 30 µl of silicone oil, which was then loaded in the cavity of a glass tube of internal diameter 2 mm and a strand of copper was forced through the other side to provide electrical connection. The surface of TH-CPE was finally made smooth over the weighing paper to serve as working electrode. Similarly a bare electrode was constructed using unmodified graphite powder for comparative purpose.

3. Results and Discussion

3.1. FT-IR spectral characterisation

The free TH and TH immobilized graphite powder were characterized by FTIR and the corresponding spectra are shown in Figure 1A. Peak at 1604 cm⁻¹ shows the vibration due to N-H bending of the primary amino group in free TH (curve a). Also, the N-H stretching vibration appears at 3339 cm⁻¹ and 3177 cm⁻¹ for free TH. The band at 1604 cm⁻¹ disappears in the spectrum of TH immobilized graphite powder (curve b) and instead a new band appears at 1585 cm⁻¹ of N-H bending mode of the resulting amide moiety. Further, the carbonyl stretching band at 1670 cm⁻¹ corresponding to the amide group appears only in the spectrum of TH immobilized graphite powder and not in the free TH spectrum showing the linkage of TH to graphite through an amide linkage.

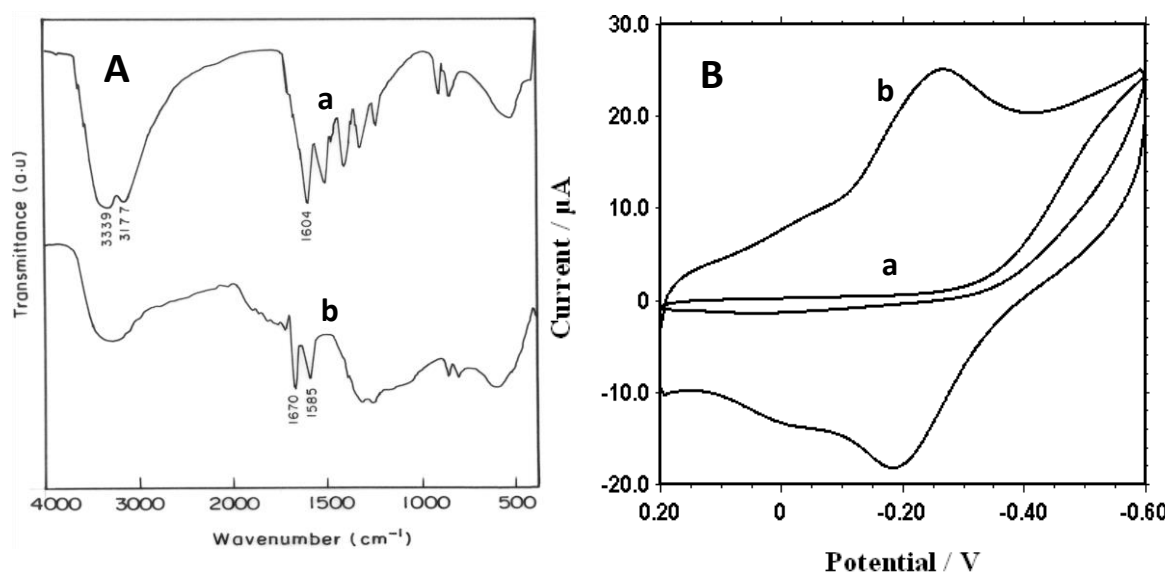


Figure 1. (A) FT-IR spectra of (a) Free Thionin (b) Thionin immobilized graphite powder (B) Cyclic voltammetric curves of [a] Bare CPE, [b] TH-CPE in NH₄NO₃ (0.1 M); Scan rate: 20 mVs⁻¹.

3.2. Electrochemical characterization of TH-CPE

TH-CPE was electrochemically characterized using cyclic voltammetry. Figure 1B (curve b) displays the cyclic voltammetric curves of TH-CPE recorded in 0.1 M supporting electrolyte solution of NH_4NO_3 (0.05 M PBS, pH 7.0) at a potential sweep rate of 20 mVs^{-1} . Thionine modified electrode exhibited a set of redox peaks at a formal potential, $E^{0'}$, of about -0.226 V [$E^{0'} = (E_{\text{pa}} + E_{\text{pc}})/2$], with the potential of cathodic peak, E_{pc} at -0.266 V and potential of anodic peak, E_{pa} at -0.186 V , corresponding to the immobilized TH mediator, on sweeping the potential in the potential window of 0.2 to -0.6 V . The separation between anodic and cathodic peaks expressed as ΔE_{p} was observed to be 120 mV . The bare CPE did not show any significant reaction under similar condition (Figure 1B, curve a).

The electrochemical response of TH-CPE in different electrolyte solutions of KNO_3 , NaNO_3 , LiNO_3 , $\text{Ba}(\text{NO}_3)_2$, $\text{Ca}(\text{NO}_3)_2$, KCl , K_2SO_4 and NH_4NO_3 shows similar voltammetric behaviour. This shows the similarity of interaction of the immobilized mediator with different electrolytes. NH_4NO_3 solution which shows comparatively better voltammogram was preferred to be the appropriate supporting electrolyte for subsequent experiments. The dependence of the electrochemical response of the TH immobilized electrode on the pH of the working solution was also tested. On varying the pH in the range 5-8, the performance of the TH immobilized CPE did not show perceptible change. However a pH of 7.00 was selected as compromise for subsequent studies.

Scan rate was varied to optimise the scan rate during the performance of the TH immobilized CPE. On increasing the scan rate from 2 to 300 mVs^{-1} both the peak currents, cathodic as well as anodic were found to enhance in a linear fashion upon increasing the square root of scan rate. The results indicate that the redox reaction involves diffusion controlled phenomenon at the surface of the working electrode.

3.3. Electrocatalytic detection of H_2O_2

Voltammetric response for H_2O_2 reduction at the TH immobilized CPE and at the bare CPE is shown in Figure 2A. The curves a and c correspond to bare CPE and TH-CPE respectively in 0.1 M supporting electrolyte solution of NH_4NO_3 (0.05 M PBS, pH 7.0) without H_2O_2 . Owing to high overpotential requirement for the reduction of H_2O_2 at most electrodes, addition of $1.89 \times 10^{-4} \text{ M}$ H_2O_2 to the electrolytic solution showed a poor behaviour with a small current response at very high potential at the bare CPE. In contrast, a prominent increase in cathodic current was observed for the same concentration of H_2O_2 at a reduced voltage of -0.266 V at the TH immobilized CPE. The enhanced current and reduced overpotential achieved during electrocatalysis at the TH immobilized CPE for H_2O_2 reduction in comparison to the bare electrode reveals the electrocatalytic effect of the immobilized TH mediator.

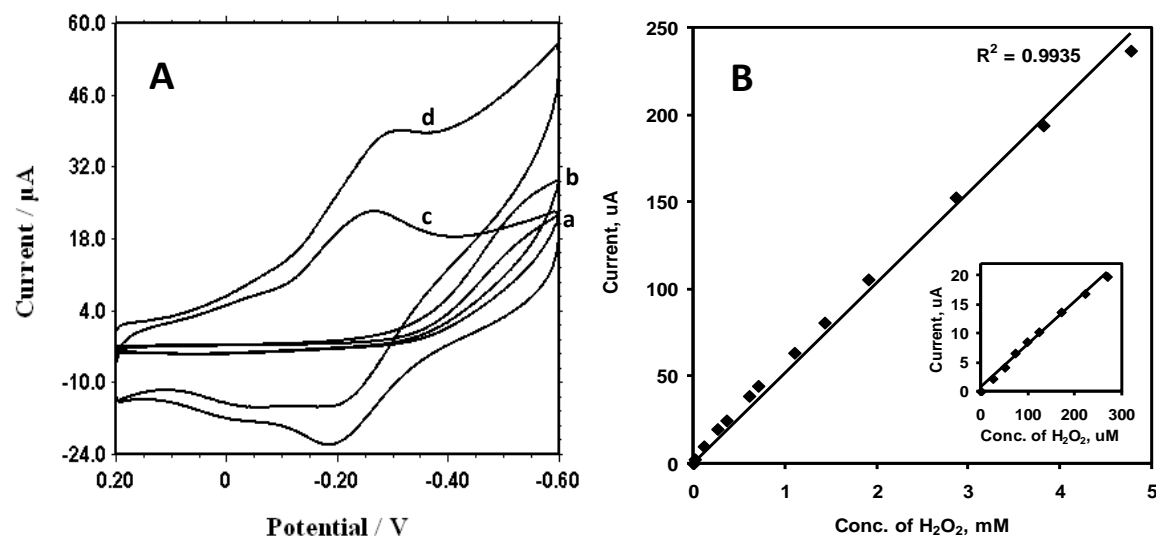
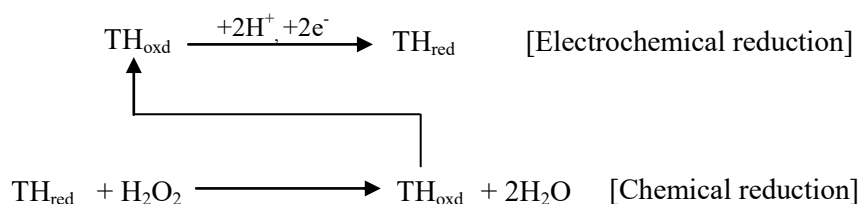


Figure 2. (A) Cyclic voltammograms of (a) bare CPE (b) 1.89×10^{-4} M H_2O_2 detection at bare CPE (c) TH-CPE (d) 1.89×10^{-4} M H_2O_2 at TH-CPE in 0.1 M NH_4NO_3 (0.05 M PBS, pH 7.0); Sweep rate: 20 mVs^{-1} (B) Calibration curve for the detection of H_2O_2 . Inset: Calibration for lower concentration of H_2O_2

Scheme 1 depicts the electrocatalytic detection mechanism of H_2O_2 at TH immobilized CPE. Initially, the immobilized TH, gets reduced with the applied potential. This reduced TH reduces H_2O_2 and gets in turn oxidised. Then the oxidised form of thionin gains electron from the electrode surface and gets regenerated to exhibit the reduction current during the same moment. These processes of H_2O_2 reduction and TH regeneration proceed in a cyclic manner, to increase the reduction current of TH mediator.



Scheme 1: Mechanism of H_2O_2 reduction with Thionin

Quantification of H_2O_2 was carried out to evaluate the modified electrode as a sensor by monitoring the cyclic voltammetric curves of the TH-CPE in the absence and presence of H_2O_2 . An increase in cathodic current was observed with increase in concentration of H_2O_2 . When the catalytic current was recorded with increasing H_2O_2 concentration, a linear trend was observed in the window of 2.46×10^{-5} M to 4.76×10^{-3} M ($R^2 = 0.9935$) (Figure 2B). The limit of detection was calculated as 1.47×10^{-5} M.

3.4. Hydrodynamic voltammetry

Hydrodynamic voltammetry studies were performed to optimize the working potential for H_2O_2 determination in flow systems. The cathodic current response was measured at constant intervals of applied potential, for 1.89×10^{-4} M H_2O_2 in the potential window of 0.2 to -0.6 V. The results from hydrodynamic voltammetry are shown in Figure 3A and are in agreement with those from cyclic voltammetry. Figure 3 B reveals that the current at the bare electrode is not significant for H_2O_2 detection. In distinction, the TH immobilized electrode shows a marked increase in current which starts even before 0 V. On increasing the cathodic potential, the response rose sharply near -0.3 V. Hence, the TH-CPE favours facile determination of H_2O_2 at lesser potentials in addition to huge sensitivity due to its electrocatalytic activity.

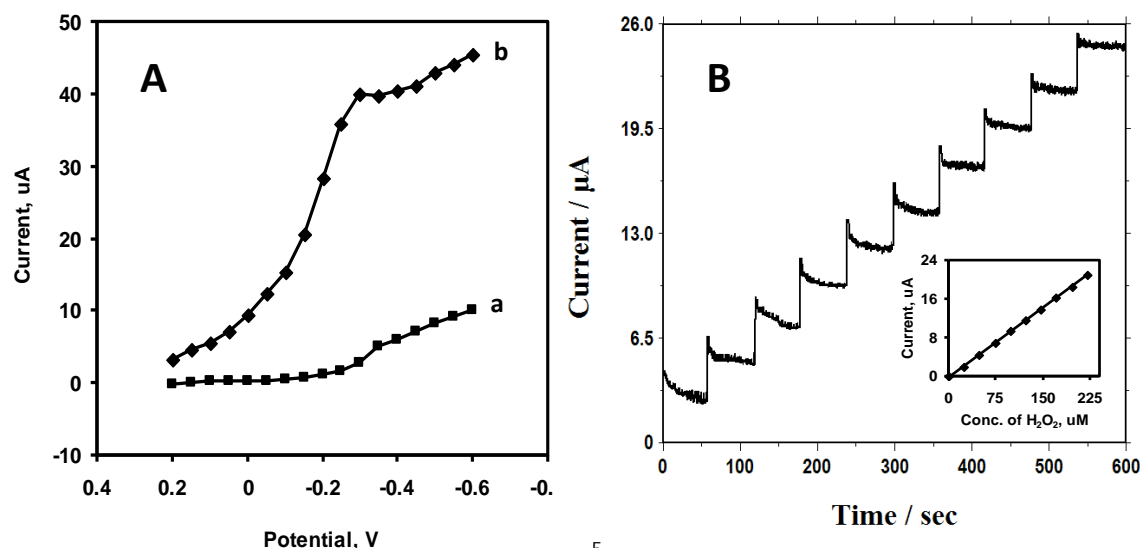


Figure 3. (A) Hydrodynamic voltammograms of 1.89×10^{-4} M H_2O_2 with [a] bare CPE, [b] TH-CPE (B) Chronoamperometric response at TH-CPE for every 0.5 ml addition of 3 mM H_2O_2 in 0.1 M NH_4NO_3 , Rotating rate: 300 rpm, Operating Potential: -0.3 V; Inset: H_2O_2 determination with the calibration graph.

3.5. Amperometric study

Chronoamperometric experiments have been carried out for the determination of H_2O_2 and optimum potential of -0.3 V was selected for this dynamic study. Amperometric response was recorded for successive injection of 3.44 mM H_2O_2 (0.5 ml aliquot) into 0.1 M NH_4NO_3 at pH 7.00. The sensor exhibited a rapid and sensitive response to the changes in H_2O_2 concentrations, which is attributed to the covalently immobilized TH. The reduction current increases with every addition of H_2O_2 and quickly reaches a stable value (<4 s) indicating the short response time of the TH-sensor. Figure 3B shows the part of the chronoamperogram recorded for H_2O_2 in the concentration domain of 2.46×10^{-5} M - 2.19×10^{-4} M and the inset displayed the corresponding calibration plot. The sensor showed good amperometric response for H_2O_2 determination upto a higher concentration of 4.52×10^{-3} M H_2O_2 . Thus TH-CPE electrode has notable and robust mediation ability and facilitates amperometric detection of H_2O_2 at a reduced potential.

3.6. Stability and reproducibility

TH-immobilized CPE exhibits good stability, reflecting the great competence of the immobilization strategy. Upon repetitive scanning from 0.2 to -0.6 V at 20 mVs^{-1} for 100 potential scans, the modified electrode exhibited no observable changes of both anodic and cathodic peak currents and potential (Figure 4A). The long-term stability of TH-immobilized CPE examined over 60 days retained 98.15 % of its initial response at the end of the study (Figure 4B). The response of the TH-CPE for the reduction of 1.89×10^{-4} M H_2O_2 for an extended length of six hours is shown as Inset to Figure 4B. It is observed that the response of TH-immobilized CPE remained almost the same, suggesting the stability of the TH-CPE under rigorous hydrodynamic conditions. This behaviour is attributable to the stable immobilization of TH mediator in the carbon paste electrode.

4. Conclusion

Graphite powder was covalently functionalised with thionin, a phenothiazine mediator and used as the electrode material to fabricate a chemically modified carbon paste electrode. A stable and surface renewable electrode with thionin covalently immobilized to the carbon surface to control leaching problems has been designed. It has been demonstrated that immobilized thionin could act as an effective catalyst for the cathodic detection of H_2O_2 at a reduced overpotential at -0.266 V. TH immobilized CPE permits convenient detection of H_2O_2 with the linear range spanning from 2.46×10^{-5} M to 4.76×10^{-3} M. The immobilization strategy proved to be efficient in retaining the water soluble mediator to the electrode surface, resulting in good operational and storage stability.

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