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Electrochemical Determination of Hydrogen Peroxide Using Silver Nanoparticle – Poly (Celestine Blue) Nanohybrid Modified Electrode

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ABSTRACT - The oxazine dye, celestine blue (CB) has been polymerised on silver nanoparticles (SNPs) modified electrode by potential cycling and the resulting SNPs/PCB film modified electrode has been studied by cyclic voltammetry, differential pulse voltammetry and chronoamperometry. FESEM, EDS and UV-Vis characterization techniques were employed to study the surface morphology. Various experimental parameters influencing the electrochemical behaviour of the modified electrode were optimized by varying the supporting electrolytes, scan rates and pH. The SNPs/PCB modified electrode showed a distinct redox peaks at -0.35V (cathodic) and -0.21V (anodic) with a formal potential of -0.28V at a scan rate of 50mV/s in 0.1M of PBS (pH 7). The SNPs/PCB modified electrodes showed excellent electrocatalytic activity toward hydrogen peroxide reduction. Voltammetric peak currents showed a linear response for H_2O_2 concentration in the range of 1.66x 10^{-5}M to 1.24 x 10^{-3}M with a sensitivity of 0.12 µA/µM and a detection limit of 5.53 x 10^{-6}M (S/N=3). The relative standard deviation for ten determinations of 1.66x 10^{-5}M H_2O_2 was 1.8%. The proposed sensor was successfully applied for selective determination of H_2O_2 in milk samples.

KEYWORDS: Hydrogen peroxide, Silver nanoparticle, Poly (Celestine blue), Amperometry.

1. INTRODUCTION

Hydrogen peroxide (H_2O_2) is a reactive oxygen species and its determination is of great interest in environmental science and in biochemistry. H_2O_2 is a major compound used in textile and paper industry, processed foods, minerals, petrochemicals and consumer products [1, 2]. Owing to the above importance, a rapid, accurate and reliable determination of H_2O_2 is of practical importance in food, pharmaceutical, clinical and environmental analysis. The development of electrochemical sensors has proved to be an effective tool for H_2O_2 determination [3, 4] owing to its low-cost, high selectivity and simplicity.

The development of nanomaterials especially metal nanoparticles play an important role in improving sensor performance. Metal nanoparticles modified electrodes show dramatically enhanced sensitivity due to its large surface area and high surface free energy. Among these materials silver nanoparticles (SNPs) show excellent catalytic activity for H_2O_2 reduction [5, 6]. Recent studies have proved that the performance of the H_2O_2 sensor depended strongly on the size, shape and distribution of SNPs on the electrode [7].

Numerous strategies or methods have been applied in the modification of electrode surfaces with redox dye molecules and their electro polymers as mediators in sensors [8]. The electroactive properties of the monomers are usually retained upon polymerisation. The immobilization method of dyes on the electrode has several drawbacks because of their low molecular weight which makes them to diffuse away easily from the electrode surface into the bulk solution which in turn affects the performance of the sensor. Polymeric dye film can efficiently overcome above mentioned problems as they impose diffusion barriers on the electrode surface. Many polymeric dyes like poly (methylene blue) [9], poly (neutral red) [10], poly (thionine) [11], poly (Celestine blue) [12,13] on the electrode surface have proven to be very stable electron transfer mediators in the detection of various analytes of biological importance.

The present work focuses on the electrochemical synthesis of poly (celestine blue) (PCB), from the oxazine dye celestine blue (CB), optimisation of the polymerisation conditions and study of the electrochemical properties of the resulting redox polymer as well as its application as redox mediator in H_2O_2 sensors. CB was electropolymerized on SNPs modified electrodes. The SNPs/PCB modified electrode was found to possess high stability and was characterised by cyclic voltammetry, differential pulse voltammetry, hydrodynamic voltammetry and chronoamperometry. Moreover, determination of H_2O_2 in a real sample was performed by the SNPs/PCB modified electrode system and satisfactory results have been obtained.

2. EXPERIMENTAL

A MATERIALS

Silver nitrate and hydrogen peroxide were purchased from Merck, India. Celestine blue dye was purchased from Aldrich, USA. Spectroscopic grade graphite rod of 3mm was purchased from Aldrich, Germany. All other reagents and chemicals used were of analytical grade and were used as received. Phosphate buffer solution (0.1 M PBS, pH 7) was prepared by mixing solutions of 0.1 M $Na_2HPO_4 \cdot 12H_2O$ and 0.1 M $NaH_2PO_4 \cdot H_2O$. Aqueous solution of H_2O_2 was prepared fresh at the time of experiments. Double distilled water was used for the preparation of all solutions.

B. CHARACTERIZATION

Field emission scanning electron microscope (FESEM) studies were carried using FESEM (SU6600, HITACHI, JAPAN) equipped with EDS. The UV-Vis spectra were recorded using a Perkin Elmer (Lambda650) spectrophotometer. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) studies and amperometric measurements were performed using CHI 660B electrochemical workstation (CH Instruments, USA). A conventional three electrode system consisting of modified electrode as a working electrode, a saturated calomel electrode (SCE) as reference electrode and platinum electrode as counter electrode linked to a personal computer for data acquisition and potential control were used. All potentials reported in this paper were against SCE. All the electrochemical measurements were carried out at room temperature.

C FABRICATION OF SNPs/PCB MODIFIED ELECTRODE

Citrate capped SNPs was prepared by the reduction of silver nitrate using tri sodium citrate **[14]** as reported earlier. The fabrication of SNPs/PCB modified electrode was carried out as follows. The surface of the graphite electrode was smoothened and dropcasted with an optimized volume of about 25 µL of SNPs and dried, followed by the electropolymerization of CB by cycling the potential between -0.6 V to 0.4V in 0.05M NH_4NO_3 solution containing 5mM of CB. For comparative studies, SNPs modified graphite and PCB modified graphite electrodes were prepared.

D. PROCEDURE

The experiments were carried out by studying the cyclic voltammetric behaviour of the SNPs/PCB modified electrode in phosphate buffer (pH 7) as supporting electrolyte in the potential range of 0.0 to -0.6 V at scan rate of 50 mV s^{-1}. Cyclic voltammetry and differential pulse voltammetry were employed to analyse the reduction of H_2O_2 by the SNPs/PCB modified electrode. This was performed by adding appropriate amount of H_2O_2 to 60 ml of supporting electrolyte of the electrochemical cell. The applicability of the SNPs/PCB modified electrode for the determination of H_2O_2 in flow systems was also studied by hydrodynamic voltammetry and chronoamperometry. Chronoamperometric studies were performed at a fixed potential of -0.4V. In the real sample analysis, standard addition method was used for analysis of spiked milk samples with aliquot amounts of H_2O_2.

3. RESULTS AND DISCUSSION

3.1. UV- Visible studies

UV-Visible spectroscopy was employed to confirm the formation of citrate capped SNPs as discussed earlier. An absorption band obtained at 420nm as shown in fig.1 is the characteristic absorption band of SNPs. This confirms the formation of SNPs within the particle size ranging from 50-100 nm which is in accordance with earlier reports **[12]**.

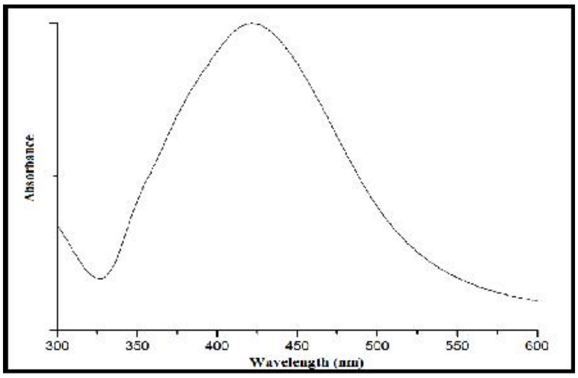

Fig.1. UV-Vis spectra of synthesized SNPs

3.2. Electrochemical polymerization of CB

Fig.2 (A & B) shows the cyclic voltammograms (CVs) of CB polymerization on bare graphite and SNPs modified graphite electrode in 0.05M NH_4NO_3 solution containing 5mM of CB. During consecutive potential cycling (20 cycles) between -0.6 V to 0.4V a pair of reversible redox peak were obtained for both bare graphite and SNPs modified electrodes respectively. The redox peak currents obtained for SNPs/PCB modified graphite electrode (fig.2B) was found to be higher than the bare graphite electrode (fig.2A). The increase in the peak currents evidences the gradual growth of the polymer film on both the electrodes. This demonstrates that the SNPs modified graphite electrode may be more favourable for the electropolymerization of CB. It may be ascribed due to the large specific surface area of SNPs modified electrode.

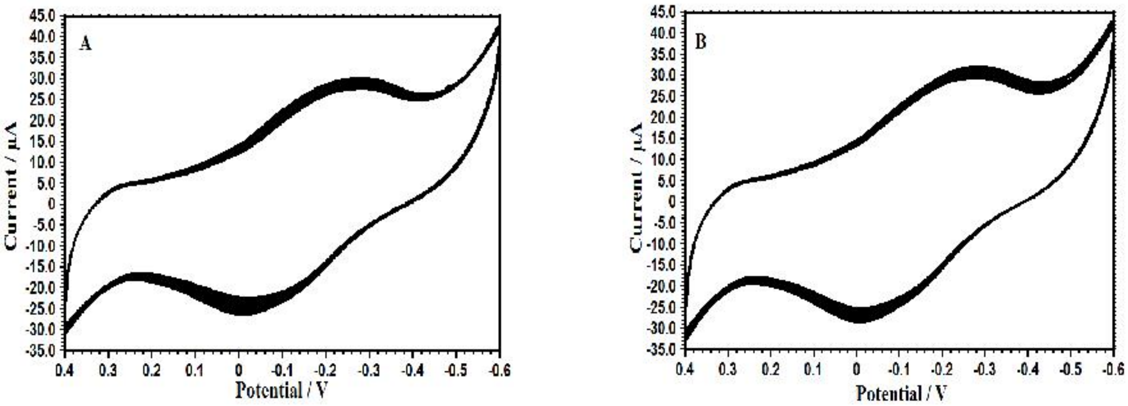

Fig.2. Electropolymerization of CB on (A) bare graphite and (B) SNPs modified graphite electrode in 0.05M NH_4NO_3 solution containing 5mM of CB, Scan rate: 50mV/s.

3.3. FESEM and EDS Analysis

FESEM analysis was used to characterize and compare the surface morphologies of the SNPs and SNPs/PCB modified electrodes. Fig. 3A corresponds to the FESEM image of SNPs modified electrode revealing the uniform distribution of the SNPs (30-80nm) on the graphite electrode. Fig. 3C represents the FESEM image of SNPs/PCB modified electrode. From the image, the polymerization of CB over the SNPs is confirmed by a dense coating of PCB over SNPs resulting in a kind of agglomeration among the particles. The corresponding EDS measurements confirm the elemental composition of the modified electrodes (fig. 3B and 3D).

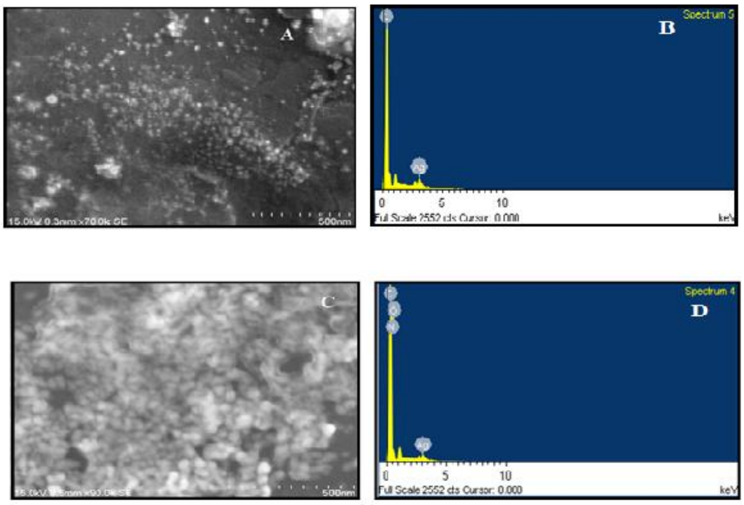

Fig. 3. FESEM and EDS images of SNPs (A & B) and SNPs/ PCB modified electrode (C & D).

3.4. Cyclic voltammetry characterization of SNPs/PCB modified electrode

The electrochemical performance of bare graphite, SNPs modified electrode, PCB modified electrode and SNPs/PCB modified electrode were investigated in a solution of 0.1M PBS (pH 7) within a potential range of 0 and -0.6V at a scan rate of 50 mVs^{-1} by cyclic voltammetry. The cyclic voltammograms of bare (curve a), SNPs modified electrode (curve b), PCB modified electrode (curve c) and SNPs/PCB modified electrode (curve d) in 0.1M PBS (pH 7) are as shown in fig.4. No characteristic peaks were obtained with the bare electrode and SNPs modified electrode (curve a and b). The SNPs/PCB modified electrode (curve d) exhibited well defined cathodic and anodic peaks at -0.35V and -0.21V with a formal potential of -0.28V when compared with PCB modified electrode. This characteristic behaviour suggests the larger electroactive surface area and excellent conductivity of SNPs/PCB modified electrode when compared with other electrodes.

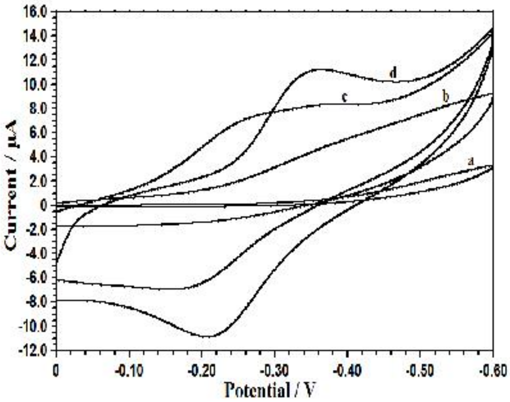

Fig. 4. Cyclic voltammograms of (a) bare (b) SNPs (c) PCB and (d) SNPs/PCB modified electrode in 0.1M PBS (pH 7), Scan rate: 50mV/s.

3.5. Effects of supporting electrolyte and pH

Various supporting electrolytes including KCl, KNO_3, LiCl, $NaNO_3$, PBS and NH_4NO_3 (0.1M) were studied in order to find the appropriate medium for effective electrochemical response of SNPs/ PCB modified electrode using CV (fig. 5). From the obtained voltammograms, a sharp and a well defined peaks for PCB was obtained with PBS (0.1M) as supporting electrolyte. Other electrolytes did not exhibit any appreciable redox behaviour for PCB film on SNPs modified electrode. Hence PBS (0.1M) solution was chosen as the background electrolyte for further studies.

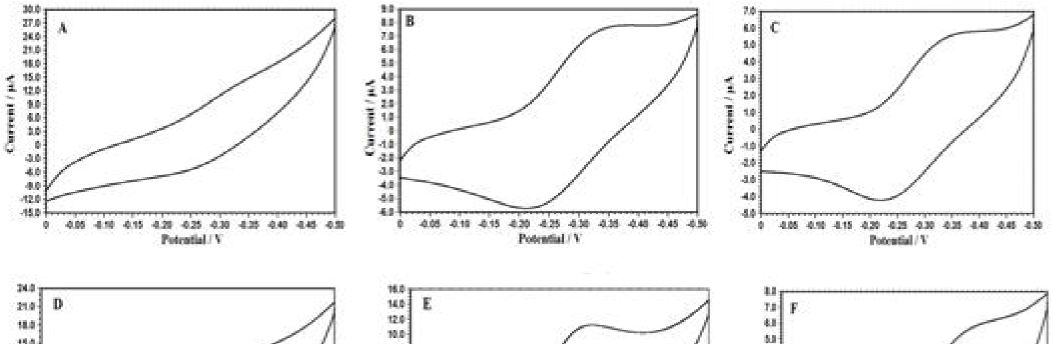

Fig. 5. Cyclic voltammograms of SNPs/PCB modified electrode in the presence of 0.1M (a) KCl (b) KNO_3 (c) LiCl (d) $NaNO_3$ (e) PBS (f) NH_4NO_3, Scan rate: 50mV/s.

The influence of pH on the electrochemical behaviour of SNPs/PCB modified electrode over a range of 3–10 was studied (fig. 6). A shift in the anodic and cathodic peak potentials towards more negative value was observed on increasing the solution pH, demonstrating a two electron–two proton process [15]. Furthermore, with increasing pH values, the peak current were found to decrease due to the desorption and dissolution of anionic forms of the dye. A maximum peak current was obtained at neutral condition and pH 7 was considered as optimum for further studies.

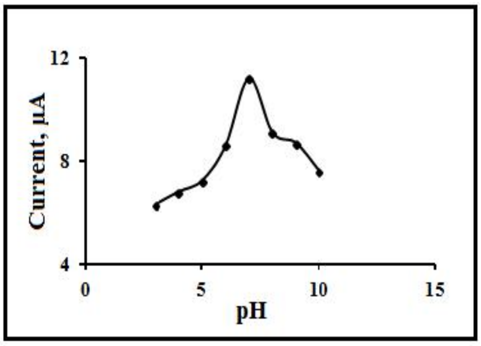

Fig. 6. Effect of pH on the peak current response of SNPs/PCB modified electrode in 0.1 M PBS solution; scan rate: 50 mV s^{-1}.

3.6. Effect of scan rate

The SNPs/PCB modified electrode was subjected to different scan rates ranging from 2-150mV/s in the presence of PBS (0.1M) solution. With increasing scan rate, the cathodic and anodic peak currents of the modified electrode were found to increase linearly (fig.7A). In the scan rate variation plot (fig. 7B), a linearity was obtained with the anodic and cathodic peak currents with increasing scan rate suggesting that there occurs a surface confined charge transfer kinetics. The surface coverage (Γ) for the SNPs/PCB modified electrode were calculated using the equation $\Gamma = Q/nFA$, where Q represents the anodic or cathodic charge under the peak in coulombs, F is the Faraday constant (96,485 C mol^{-1}), A is the surface area of the electrode and n is the number of electrons transferred [16]. The calculated Γ values for SNPs/PCB modified graphite electrode was 3.69×10^{-10} mol/cm^2 and that of PCB modified electrode was recorded as 1.72×10^{-10} mol/cm^2. The differences in the Γ values indicate that the SNPs have offered a stable platform for immobilizing PCB.

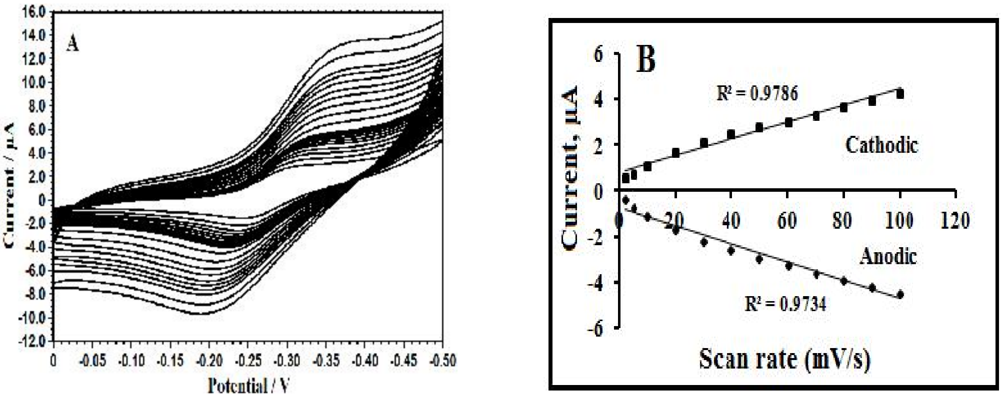

Fig.7. (A) Cyclic voltammograms of SNPs/PCB modified electrode at different scan rates; (B) Dependence of peak current vs. scan rate. Electrolyte: 0.1M PBS (pH 7); Scan rate: 50mV/s .

3.7. Electrocatalytic reduction of H_2O_2 at SNPs/PCB modified electrode

Electrochemical experiments were carried out to investigate the catalytic activity of H_2O_2 at SNPs/PCB modified electrode. Fig.8A gives the voltammetric response of the bare graphite, PCB modified electrode, SNPs/ PCB modified electrode in the absence (curve a, c, e) and in the presence (curve b, d, f) of 8.26×10^{-5} M H_2O_2. The bare graphite and PCB modified electrode were found not to exhibit appreciable catalytic activity (curve b, d). The SNPs/PCB modified electrode exhibited excellent catalytic activity towards H_2O_2 reduction with a significant increase in the cathodic peak current. The effect of the solution pH on the catalytic behavior of SNPs/PCB modified electrode was studied in the range of 3-10. It was observed that at a neutral pH 7 maximum current was recorded. At higher pH, the peak currents were found to decrease. So pH 7 was chosen as optimum condition for subsequent determinations. A siginificant increase in the cathodic peak currents were observed with successive addition of H_2O_2. This indicates that the reduction of H_2O_2 was effectively electrocatalysed by SNPs/PCB modified electrode. A plot of catalytic current Vs concentration of H_2O_2 (fig. 8B) showed linearity ($R^2 = 0.9982$) in the concentration range from 1.66×10^{-5} M to 1.24×10^{-3}M with a detection limit of 5.53×10^{-6} M (S/N = 3). The sensitivity for the determination was obtained as 0.12µA/µM. The RSD for ten repetitive additions of 1.66×10^{-5}M of H_2O_2 was 1.8%.

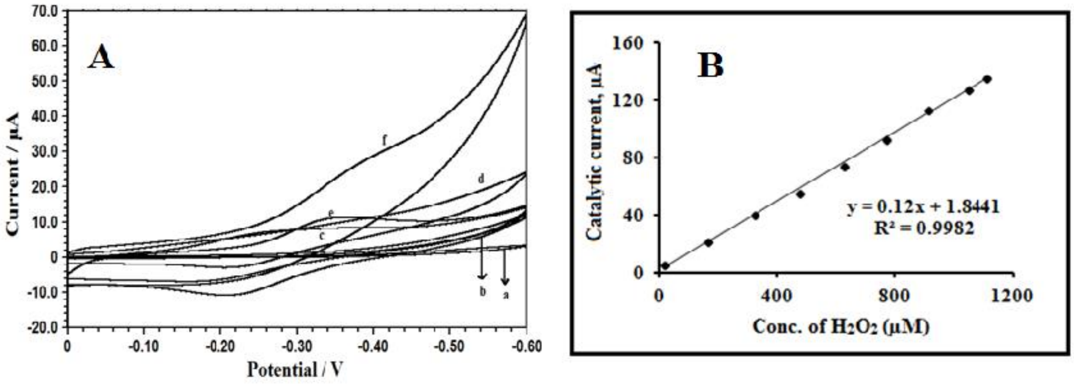

Fig. 8. (A) Cyclic voltammograms of bare, PCB and SNPs/PCB modified electrode in the absence (a, c, e) and presence (b, d, f) of 8.26 $\times10^{-5}$ M H_2O_2 (B) Calibration plot of catalytic current vs. concentration of H_2O_2; Electrolyte: 0.1M PBS (pH 7), Scan rate: 50mV/s.

Differential pulse voltammetric (DPV) studies were also carried out to examine the applicability of SNPs/PCB modified electrode towards the reduction of H_2O_2. Fig.9 shows the DPV response in the presence of various concentrations of H_2O_2. A linear increase in the peak current was observed for successive additions (0.2ml) of H_2O_2 in 0.1M PBS (pH 7). These results demonstrated that the SNPs/PCB modified electrode showed efficient electrocatalytic response towards the reduction of H_2O_2.

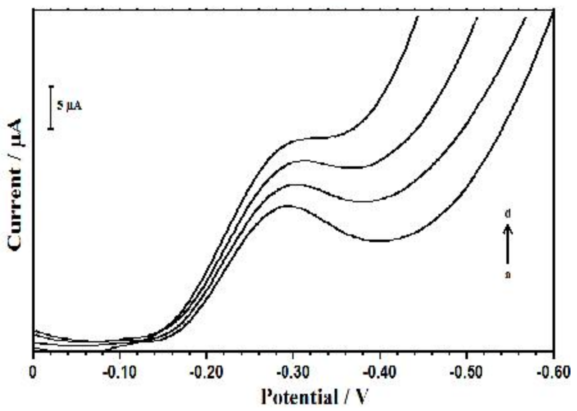

Fig. 9. DPV response of SNPs/PCB modified electrode for the successive additions of 0.2ml of 0.01M H_2O_2 (a to d). Electrolyte: 0.1M PBS (pH 7), Scan rate: 50mV/s.

3.7.1. Hydrodynamic voltammetry

The applicability of the SNPs/PCB modified electrode towards reduction of H_2O_2 under dynamic conditions was tested using hydrodynamic voltammetry technique. The current response in the range of 0 to -0.6V was recorded at a stirring rate of 300 rpm. Fig. 10 gives the hydrodynamic response of bare (curve a) and SNPs/PCB (curve b) modified electrode in the presence of 8.26×10^{-5} M H_2O_2. It can be observed that the modified electrode (curve b) exhibited maximum current response for H_2O_2 at a potential of -0.35V even under dynamic conditions whereas the bare electrode (curve a) did not show any considerable current response. Based on the hydrodynamic studies, an operational potential of -0.4V was chosen for the chronoamperometric determination.

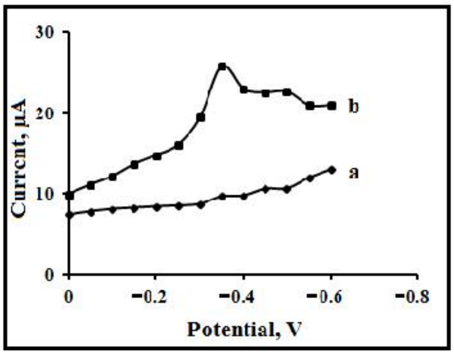

Fig. 10. Hydrodynamic voltammograms of (a) bare (b) SNPs/PCB modified electrode in presence of 8.26×10^{-5} M of H_2O_2. Electrolyte: 0.1M PBS (pH 7), Scan rate: 50mV/s, under stirring condition.

3.7.2. Chronoamperometry

The performance of the SNPs/PCB modified electrode for H_2O_2 reduction was evaluated by amperometric measurements at an applied potential of -0.4V. Fig.11A gives a current–time plot for the SNPs/PCB modified electrode under the optimized fixed potential of -0.4V for successive additions of 0.2ml of 0.01M H_2O_2. A steady state condition was reached within 3 s. Fig. 11B gives the calibration plot of catalytic current versus concentration of H_2O_2. The SNPs/PCB modified electrode showed good linear relationship in the concentration range from 3.32×10^{-5} M to 2.91×10^{-4} M with a correlation co efficient of 0.996. This behaviour of the SNPs/PCB modified electrode under dynamic conditions makes it applicable for the determination of H_2O_2 even in flow systems.

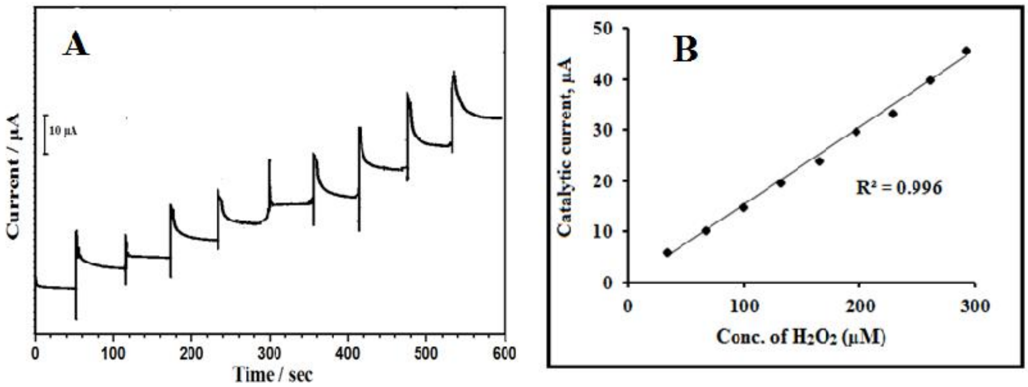

Fig. 11. (A) Chronoamperometric response for H_2O_2 at SNPs/PCB modified electrode for each 0.2ml addition of 0.01M H_2O_2. Applied potential: -0.4V, (B) Calibration plot for amperometric determination of H_2O_2; Electrolyte: 0.1 M PBS solution (pH 7), Scan rate: 50 mV/s, under stirring condition.

3.8. Interference studies

The anti interference ability of the SNPs/PCB modified electrode in the electrochemical determination of H_2O_2 was studied in the presence of various analytes like ascorbic acid, uric acid and acetic acid. The current response was recorded in the presence of these species with H_2O_2 in 1:2 ratio. The results indicated that these species did not exhibit any observable interference in the determination of H_2O_2 which may be attributed to the low potential determination of H_2O_2.

3.9. Analytical applicability

Commercially available two different branded milk samples were assayed to demonstrate the practical use of the proposed sensor. The samples were spiked with two different concentrations of H_2O_2 and the recoveries were determined. The average recoveries were calculated as 99.5% and 100% respectively. A blank milk sample was also tested in the same way and it did not show any signal. The relative standard deviation (n = 5) was calculated and the results are tabulated (table.1).

Table. 1: Determination of H_2O_2 in milk sample

Sample No.	**Added (µM)**	**Found^a (µM)**	**RSD**	**Recovery (%)**
1	20 40	19.7 40.02	1.6 1.4	98.5 100
2	20 40	20.1 40.02	2.1 2.3	100.5 100

[a] = The values are average of five replicate measurements

3.10. Stability of the SNPs/PCB modified electrode

The stability of the SNPs/PCB modified electrode was studied by repetitive cycling (100 cycles) of the modified electrode in 0.1M PBS at a scan rate of 50mV/s (fig. 12). The curves a and b represents the 1st and the 100th potential cycle with negligible decrease in the cathodic peak current and anodic peak currents. This confirms that the SNPs have provided a stable platform for the polymerization of CB and also shows the reproducibility, reliability and applicability of the modified electrode.

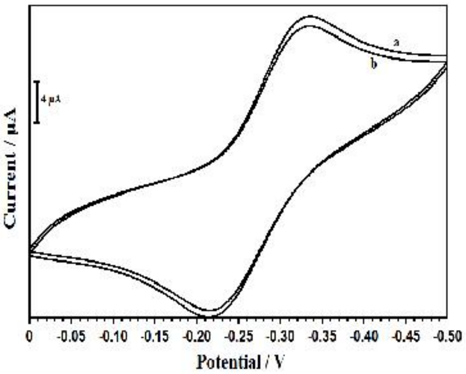

Fig. 12. Cyclic voltammograms of (a) 1st and (b) 100th cycle of SNPs/PCB modified electrode in 0.1 M PBS, Scan rate: 50 mV s^{-1}.

IV. Conclusion

A new electrode based on SNPs/PCB modified electrode is prepared, characterized and utilized for the electrochemical sensing of H_2O. The SNPs synthesized and the polymerization of CB on the SNPs modified electrode was confirmed by UV–visible absorption spectroscopy and FESEM analysis. The electrode has a higher electrochemical activity toward the reduction of H_2O_2. The modified electrode exhibited very good linearity in the concentration range of 1.66×10^{-5} M to 1.24×10^{-3}M with a detection limit of 5.53×10^{-6} M (S/N = 3) and sensitivity as 0.12µA/µM. The electrode was successfully applied for the determination of H_2O_2 in real samples. The modified electrode exhibited good reproducibility and long term stability.

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References

[1] H.Y. Wang, S.M. Park, “Polypyrrole-Based Optical Probe for a Hydrogen Peroxide Assay”, Anal. Chem., vol. 79, pp. 240-245, 2007.
[2] Y.F. Yang, S.L. Mu, “Determination of hydrogen peroxide using amperometric sensor of polyaniline doped with ferrocenesulfonic acid”, Biosens. Bioelectron., vol. 21, pp. 74-78, 2005.
[3] S. Zong, Y. Cao, Y. Zhou, H. Ju, “Zirconia Nanoparticles Enhanced Grafted Collagen Tri-Helix Scaffold for Unmediated Biosensing of Hydrogen Peroxide”, Langmuir, vol. 22, pp. 8915-8919, 2006.

[4] S.A. Kumar, S.-M. Chen, “Electrocatalytic reduction of oxygen and hydrogen peroxide at poly(p-aminobenzene sulfonic acid)-modified glassy carbon electrodes”, J. Mol. Catal. A: Chem., vol. 278, pp. 244-250, 2007.

[5] K. Cui, Y. Song, Y. Yao, Z. Huang, L. Wang, “A novel hydrogen peroxide sensorbased on Ag nanoparticles electrodeposited on DNA-networks modified glassycarbon electrode”, Electrochemistry Communications, vol. 10, pp.663-667., 2008.

[6] C.M. Welch, C.E. Banks, A.O. Simm, R.G. Compton, “Silver nanoparticle assem-blies supported on glassy-carbon electrodes for the electro-analytical detectionof hydrogen peroxide”, Anal Bioanal Chem., vol. 382, pp. 12-21, 2005.

[7] H. Chen, Z. Zhang, D. Cai, S. Zhang, B. Zhang, J. Tang, Z. Wu, “A hydrogen peroxidesensor based on Ag nanoparticles electrodeposited on natural nano-structureattapulgite modified glassy carbon electrode”, Talanta, vol. 86, pp. 266-2 70, 2011.

[8] R. Pauliukaite, M.E. Ghica, M.M. Barsan, C.M.A. Brett, “Phenazines and Polyphenazines in Electrochemical Sensors and Biosensors”, Anal. Lett., vol. 43, pp. 1588-1608, 2010.

[9] D. Benito, J.J. Garcia-Jareno, J. Navarro-Laboulais, F. Vicente, “Electrochemical behaviour of poly(neutral red) on an ITO electrode”, J. Electroanal. Chem., vol. 446, pp. 47-55, 1998.

[10] G. Inzelt, E. Csahok, “Electrochemical Quartz Crystal Microbalance Studies of the Formation and Redox Behavior of Poly(Neutral Red) Electrodes”, Electroanalysis, vol. 11, pp. 744-748, 1999.

[11] T. Ohsaka, K. Tanaka, K. Toduda, “Electrocatalysis of poly(thionine)-modified electrodes for oxidation of reduced nicotinamide adenine dinucleotide”, J. Chem. Soc., Chem. Commun., pp. 222-224, 1993.

[12] N.S. Sangeetha, Sundara Ramaprabu and **S. Sriman Narayanan**, “Poly (Celestine Blue) - Graphene Oxide Nanohybrid modified electrode for Amperometric determination of Hydrogen peroxide”, Graphene, vol. 1, pp. 136-141, 2014.

[13] N.S. Sangeetha and S. Sriman Narayanan, “Hydrogen peroxide sensor based on carbon nanotubes- poly (Celestine Blue) nanohybrid modified electrode”, Advanced Materials Research, vol. 938, pp 263 – 268, 2014.

[14] Z.S. Pillai, P.V. Kamat, “What Factors Control the Size and Shape of Silver Nanoparticles in the Citrate Ion Reduction Method?”, J. Phys. Chem. B, vol.108, pp. 945-951, 2004.

[15] Abdollah Noorbakhsha, Abdollah Salimi, “Amperometric detection of hydrogen peroxide at nano-nickel oxide/thionine and celestine blue nanocomposite-modified glassy carbon electrodes” Electrochimica Acta, vol. 54, pp. 6312-6321, 2009.

[16] F. Ricci, A. Amine, D. Moscone, G. Polleschi, “Prussian Blue Modified Carbon Nanotube Paste Electrodes: A Comparative Study and a Biochemical Application”, Anal. Lett., Vol.36, pp. 1921-1938, 2003.