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Polyaniline/Multiwall Carbon Nanotubes/Starch Nanocomposite Material and Hemoglobin Modified Carbon Paste Electrode for Hydrogen Peroxide and Glucose Biosensing

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Abstract This paper describes the application of novel nanocomposite material Polyaniline/Multiwall Carbon Nanotubes/Starch (designated as PCS) as a good electrode material for electrochemical sensing. The developed ternary composite system has manifold interactions and synergistic improved properties - high surface area, good electro-activity, conductivity, stable dispersion, biocompatible, hydrophilic and hydrophobic regions, multifunctional, and nanoturmeric shape morphology. Hydrogen peroxide biosensor was fabricated as a model system using PCS and hemoglobin (HB) modified carbon paste electrode. PCS and HB compatible and effectively communicate with each other, facilitate the charge transfer (potassium ferricyanide used as electroactive marker). Thus, PCS and HB (in combination) is a good electrode material for sensitive and selective detection of peroxide. The developed biosensor showed a linear range (0.1 mM - 5 mM, $R^2 = 0.9975$), limit of detection (0.032 mM), sensitivity (76.43 µA/mM cm^2) and long-term storage and stability. This material could be used a platform to develop some other sensors by using other redox enzymes. As a model, we incorporate glucose oxidase to detect Glucose. Thus, the favorable properties indicate that the proposed material system suitable for low-cost sensor strips.

Keywords: Biosensor; Polyaniline; Multiwalled Carbon Nanotubes; Starch; Composite material

1. Introduction

In recent years, a variety of functional nanocomposite materials have been widely used for biosensor systems, due to its small size, large surface-to-volume ratio, high surface activity, selective interaction and excellent catalytic activity. The electrode material used for electrochemical sensor applications should have good transport of charge and mass, better electroactivity, biocompatible, uniform dispersion and good adhesion to the electrode surface, operational and storage stability. A biosensor device has a biological element (such as enzyme, tissue, cell, antibody, DNA, and protein) integrate with the transducer for selective interaction and sensing of an analyte. Enzymatic bio-sensors are the most explored due to the inherent selective catalytic activity of the enzyme for a specific analyte and commercial availability of enzymes. However, enzymes are expensive bio-molecules and its purification and processing required additional attention, its activity and structural integrity are very sensitive to chemical and physical parameters (such as temperature, pH, humidity, ionic detergents, and other chemicals). The stability of enzymes on the transducer surface is a great challenge in front of biosensor technologists because the poisoning of enzyme molecules leads to poor analytic performance. Enzymatic biosensors fabrication involves complicated and multi-step immobilization methods. To overcome the challenges, stable proteins and chemical catalyst have been used to develop chemical sensors [1, 2].

H_2O_2 is used as for disinfection, odour control, antiseptic and bleaching. Many peroxide sensors have been developed using different biomolecules and transition metal complexes as a catalyst, such as - HRP, Catalyse, Myoglobin, Hemoglobin, Prussian blue and other nanomaterials. In the biological system, the control productions of

hydrogen peroxide are important for cell signalling and kill pathogens. However, an uncontrolled production of hydrogen peroxide by enzymatic reactions in the disease conditions. Monitoring peroxide concentration is used as a tool to measure the progress of enzymatic reactions and to detect other important biomarkers. Many methods have been developed to estimate peroxide concentration, however a reliable, rapid and economical method its analysis has significant importance in industrial and academic chemical analysis [3, 4, 5, 6].

Hemoglobin (HB) is a stable, cost-effective protein molecule capable to interact with oxygen, carbon dioxide, and effective biocatalyst H_2O_2 reduction. Hemoglobin (molar mass of ~76 kDa) is an assembly of four globular metalloprotein subunits, found in the red blood cells of all vertebrates. Each subunit is composed of a protein chain tightly associated with a non-protein prosthetic heme group (consist pyrrole molecules cyclically linked together by methine bridges) with the iron ion bound in the center - known as a porphyrin). The centre iron ion coordinates with the four nitrogen atoms (lie in one plane). The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules and iron is the active binding site. When oxygen is not bound, water molecule fills the site through weak bonds. Even though carbon dioxide is also carried by hemoglobin bound to the protein chains, it does not compete with oxygen for the iron-binding positions. However, the electron transfer reactivity of HB has a physiological disadvantage; the electroactive centre is hidden within the protein sheath so signal capture during the biological recognition event at the electrode surface is a challenge. A low-cost peroxide sensor could be easily developed by immobilizing HB on the electrode surface along with redox mediator [7, 8, 9, 10, and 11].

A hybrid material system has superior properties and it is a good approach to overcome the individual deficiency of materials. Polyaniline based composite materials have been extensively used for electrochemical sensor applications due to inexpensive monomer, easy synthesis, tunable properties, effective mass/ charge transfer, environmental stability, variable oxidation state, nanoarchitecture, good electroactivity, and wide range conductivity. Conductivity and electro-activity of the PANI greatly depend upon the degree of oxidation and protonation. The positive charge on the oxidized form of polymer chains was compensated by anionic dopants. The microcavities of composite material could host the immobilized bio-molecules and other nanoparticles [12, 13, 14, 15, 16, 17, and 18].

Starch $(C_6H_{10}O_5)_n$ is a naturally abundant polysaccharide, produced by green plants as an energy store and used in human diets. It comprises glucose monomers joined together by α 1, 4 linkages, contain 20 to 25% amylose and 75 to 80% amylopectin by weight. It is white, tasteless and odorless amorphous micro-granular powder. Starch is partially soluble, particularly in cold water; however, it gives hydrogel on boiling with warm water. Starch has been used to develop environment-friendly biocompatible material for a variety of biological and technological applications. Numerous hydroxyl group and sugar units could maintain the stability of biomolecules within starch-based materials [19, 20, and 21].

A trace amount of conducting nanoparticle could drastically be improved the characteristics of the composite material, particularly large surface area, higher strength, good electrical, and electrocatalytic properties. Carbon nanotubes have emerged as very versatile interesting conducting nanofiller and extensively used to modify for composite preparation and to modify electrode surface. CNT is a suitable candidate for many

technological applications due to high conductivity, chemical stability, good strength with phenomenal unique structure and very high surface-to-volume ratio. Numerous research articles have described the important characteristics and applications of CNTs based nanocomposite materials [22, 23, and 24].

PANI-polysaccharide composite systems have to be proven promising material for many applications. In our previous communication, we have extensively studied the morphological, spectroscopic, thermal, and electrochemical characteristics of PANI, MWCNTs, and Starch based binary and ternary composite materials. The ternary material gives stable water dispersion, due to the presence of hydrophilic/hydrophobic regions, porous architecture, and numerous hydroxyl functional groups, make it an ideal candidate for the formulation of electroactive conducting ink for screen printed sensor strips. Starch is non-conducting and nonelectroactive polymers which hinder the inter-granular charge transfer, but it maintains the positive charge of polyaniline chain by suppressing the deprotonation process. Starch could improve the electro-activity by stabilizing the positive charge of PANI and improve compatibility for a biocatalyst. MWCNTs act as a conducting nanowire for better charge transfer. It improves inter-grains charge mobility and increases the surface area of composite material [25, 26]. The properties of PANI/MWCNTs/Starch composite material attested its application for bio-sensors. In this paper, the developed composite material use to fabricate electrochemical sensing of hydrogen peroxide, HB used as a biocatalyst. Hydrogen peroxide is generated during many enzymatic redox reactions, monitoring of hydrogen peroxide could be used as a tool to detect other analytes using suitable biocatalysts. In this paper, we have used glucose oxidase (along with PCS+ HB) to detect glucose, as a model system.

2. Materials and methods

2.1.Reagents and materials

The chemicals and reagents required for this research work were procured from the local vendor; Hemoglobin, Glucose oxidase, Glucose (CDH Chemicals), H_2O_2 and Aniline (Merck), Ammonium peroxidisulfate, HCl and Acetone (Qualigens Fine Chemicals India), Starch (Loba Chemie). MWCNTs prepared by chemical vapor deposition technique [NANOCTC 7000, FRAHN, HOFFER IPA, July 2013, MRTIRMATIS] and purified by boiling with concentrated HCl. Phosphate buffer solution (PBS, 0.1 M) was prepared with stock solutions of Na_2HPO_4 and NaH_2PO_4, pH was adjusted by 0.1 M NaOH/HCl. Graphite powder and Nujol oil were purchased from Sigma-Aldrich, USA. Double distilled water was used to make all solutions. Aniline was vacuum distilled at 182°C, before its use. Starch was used without any further purification and all the solutions were prepared using double distilled water.

2.2. Instrumental analysis

Electrochemical Analyzer/Workstation model 600D Series, CH instruments, USA was used to study different electrochemical analysis, such as cyclic voltammetry, chronoamperometry, chronocoulometry and electrochemical impedance spectroscopy (EIS). The three electrode systems consist a platinum wire, Ag/AgCl (saturated KCl) and modified carbon paste (glass capillary used as electrode body) as the counter, reference, and working electrode, respectively. The pH of buffer solutions was monitored by using a sistronics digital 335 pH meters (Metrohm, Switzerland) at room temperature. Scanning electron microscope (SEM) observations were performed by using FEI Quanta 200, at suitable voltages and magnifications. TEM examination was made using FEI, TECHNAI 20G2 electron microscope at an accelerating voltage of

200KV. HR-HRSEM observations were performed, using NOVA NANOSEM. Electrochemical studies were carried out, using CH600D electrochemical workstation, CH Instruments U.S.A., using a three electrode configuration. Modified carbon paste, Ag/AgCl (saturated with KCl) and Pt wire were used as working, reference and counter electrodes, respectively. 10 mg composite material and 2 mg HB was mixed with 100 mg fine quality graphite powder to form a homogeneous paste with 10 µl Nujol oil. A little amount of this paste was applied to the tip of the capillary electrode. For glucose analysis, 2 mg glucose oxidase was mixed uniformly in the carbon paste.

3. Results and discussion

3.1. Mechanism of peroxide sensing

Generally, multi-component systems are multifunctional in nature and have complex interactions which generate certain synergistically improved characteristics. In the proposed system, the three different materials were integrated with each other and performed a specific role during the electrochemical sensing of hydrogen peroxide. PCS exhibited good electroactivity and mediate charge transfer from redox center of hemoglobin. Figure-1 shows the schematic diagram of the charge transfer mechanism and molecular structures of individual constituents of the developed ternary nanocomposite material. Hemoglobin was used as cost-effective bio-catalyst to reduce hydrogen peroxide. PCS nanoparticles mediated the charge transfer at a lower potential. PCS particles interact with hemoglobin through weak hydrogen bonding. Polyhydroxyl functionality of starch provides biocompatible microenvironments, which maintains the conformational integrity and catalytic activity of HB molecule. HR-SEM and TEM images confirmed that the PCS nanocomposite has porous nano-turmeric shape structure. Morphological analysis and EDX elemental analysis of PCS is given as

supplementary information (c.f. S1). MWCNT were uniformly distributed within the composite as conducting nano fillers for charge transfer and prevents. MWCNTs are a nonelectroactive carbonaceous nanomaterial but improve the catalytic activity of material due to higher conductivity and larger surface area. The cathodic current was increased with subsequent addition of H_2O_2 in the electrolyte, attributed to the electrocatalytic reduction of peroxide by PCS-HB, represented as follows,

$$H_2O_2 + HB_{(red)} \rightarrow 2H_2O + HB\text{-}O_{(Ox)} \quad (1)$$

$$HB\text{-}O_{(Ox)} + PCS_{(Red)} \rightarrow HB_{(red)} + PCS_{(Ox)} + H_2O \quad (2)$$

$$PCS_{(Ox)} + 2e^- + 2H^+ \rightarrow PCS_{(Red)} \quad (3)$$

Net reaction is

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (4)$$

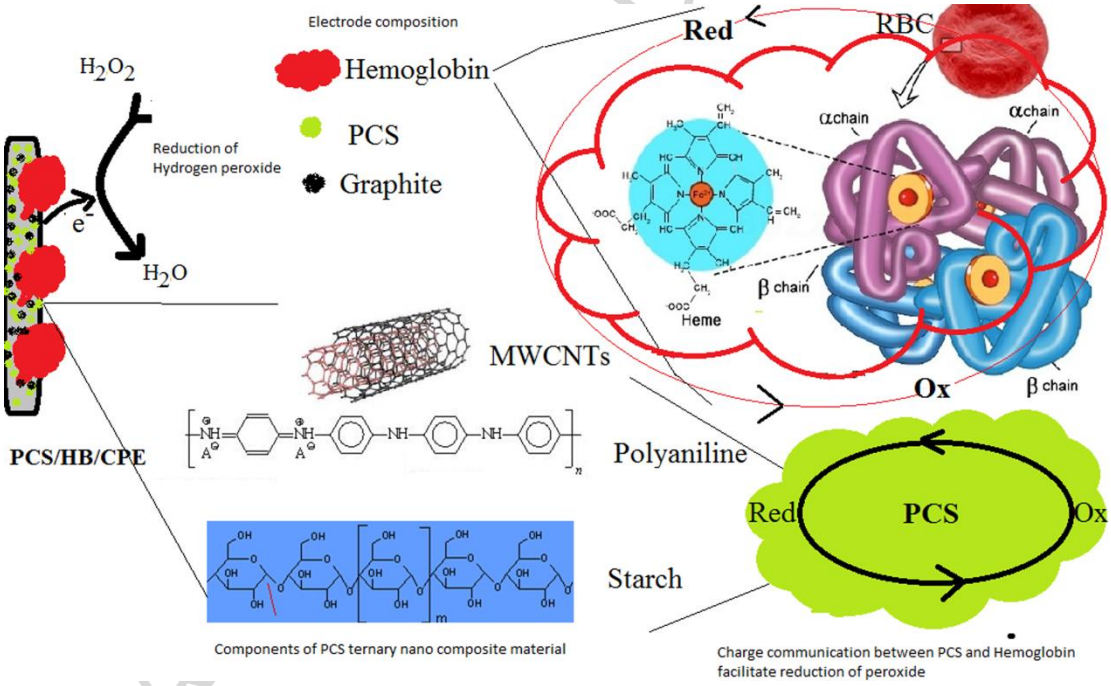

Fig. 1 Schematic diagram of functioning of peroxide biosensor, and molecular structures of MWCNTs, starch, polyaniline, and hemoglobin

3.2. Cyclic Voltammetry

Cyclic voltammetry is a primary electrochemical technique which provides details of oxidation/reduction states, mechanisms of electrochemical reactions and rate

of heterogeneous electron transfer. These valuable information has been derived from the analysis of Cyclic voltammogram's parameters, i.e. cathodic and anodic peak current (I_c, I_A), peak potentials (E_C, E_A), the formal potential (E^0) and peak-peak potential difference (ΔE). On comparing voltammograms of ternary and binary systems, we observed that PANI/MWCNTs composite material has higher peak current than pristine PANI due to the large surface area, linear alignment of chain and internal charge transfer by conducting nano-fillers "MWCNTs". Electroactivity and conductivity of PANI are closely associated with doping/dedoping level of protons and associated anions. The CV response depends on the oxidation states, morphology, surface area, and spatial chain arrangement of the PANI. In the acidic solution (at lower pH), three redox couples were observed, which associated with doping/dedoping of proton and anions. PANI/Starch composite showed lower peak current than pristine PANI attributed to the hindrance in charge transfer insulation caused by starch granules, as reported earlier [25].

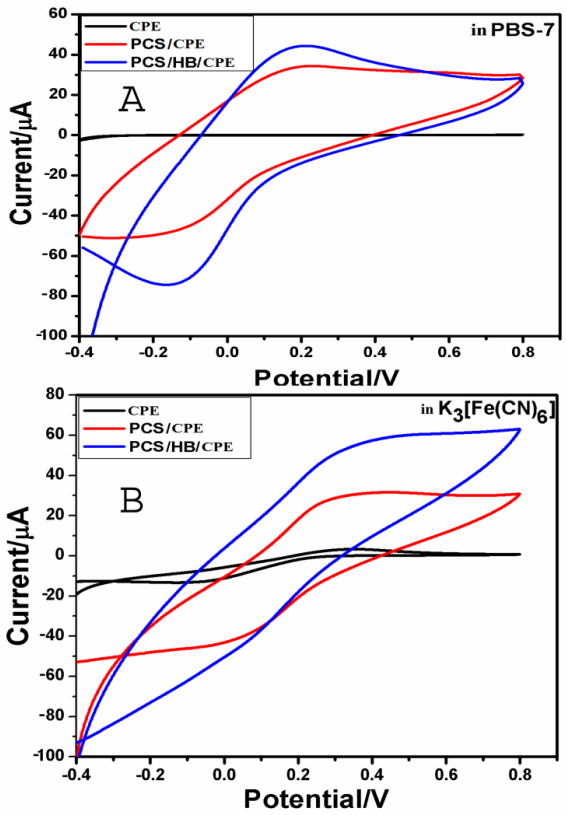

Fig. 2 Cyclic voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE, at scan rate of 20 mV/s (A) in 0.1M PBS (pH 7.0) (B) in presence of Potassium Ferricyanide solution in 0.1M PBS-7

The cyclic voltammograms of PCS and PCS-HB modified electrodes were measured in 0.1 M PBS-7, at the scan rate 20 mV/s (fig. 2A). During the synthesis of PANI in dilute HCl, chloride ions compensate the positive charge of the chain. In pristine PANI, small size chloride ions could easily leach out from the polymer matrix, results in the loss in electro-activity at neutral or higher pH. The positive charge on PANI was destabilized on de-protonation and lowers the charge transfer capability. The combined effect of MWCNTs and starch result in improved electroactivity of PCS than pristine PANI. PCS showed good electrical, electrochemical and surface properties due to reversible conversion between the leucoemeraldine and emeraldine oxidation state of

PANI (starch and MWCNTs are not electro-active materials). We inferred that with HB induces fast electrode reaction in a more reversible manner, i.e. lower formal potential and lower peak-peak potential difference.

	I_c	E_c	I_A	E_A	E^0	ΔE
	µA	**(mV)**	**µA**	**(mV)**	**(mV)**	**(mV)**
PCS/CPE	-49.46	184	33.45	207	196	392
PCS/HB/CPE	-74.23	-155	44.34	201	178	356

Cyclic voltammograms for bare CPE, PCS/CPE, PCS/HB/CPE were also measured in the presence of Potassium ferricyanide (used as redox marker), to demonstrate the electron transfer process. The higher redox current with hemoglobin and composite material support the fact that HB is compatible with composite material and facilitated the electron transfer (fig. 2B). PCS/HB modified electrode showed a steep increase in current rather than a sharp cathodic peak.

	I_c	E_c	I_c	E_a
	(µA)	**(mV)**	**(µA)**	**(mV)**
Bare-CPE	-12.45	-55	3.67	315
PCS -4/CPE	-45.50	48	28.75	308
PCS/HB/CPE	-92.34	~ -400	57.08	383

Cyclic voltammograms were recorded at different scan rates, 10 to 450 mV/s, in 0.1 M PBS (pH 3) solution. We used PBS solution of lower pH as supporting electrolyte, in order to suppress the effect of deprotonation process. In general, two redox couples were observed in the solution at lower pH (associated with doping/ dedoping of protons and anions). Peak current was directly proportional to the square root of the scan rate, attributed to diffusion control electrode process (fig. 3). On the

modified electrode with PCS + HB, the formal potential first redox couple (E_1^0) shifts toward negative potential and ΔE increases.

	E_1^0 (mV)	ΔE (mV)	E_2^0 (mV)	ΔE (mV)
PCS/CPE	-151	156	324	362
PCS/HB/CPE	-302	297	325	450

The PCS+HB modified electrode has a large potential difference in more acidic solution than the neutral solution, could be explained on the basis of sluggish electrode kinetic due to little deterioration of HB.

On comparing CV (100 mV/s and 450 mV/s), at higher scan rate the anodic peak shifts towards higher potential and cathodic peak shifted towards lower potential indicate irreversibility of the reaction and current rises due to greater diffusion of ions. Thus, we inferred that the blend of PCS composite material and HB exhibit better electron transfer reaction than individually modified electrodes (PCS-CPE, HB-CPE). Variation in the cyclic voltammograms strongly suggests the existence of good communication between composite material and hemoglobin.

	Scan rate (mV/s)	I_{a1} (µA)	I_{c1} (µA)	I_{a2} (µA)	I_{c2} (µA)
PCS/CPE	100	67	-97.5	107	-73.2
	450	206	-234	269	-179.8
PCS/HB/CPE	100	158	-195	217	168

450 326 -368 434 -296

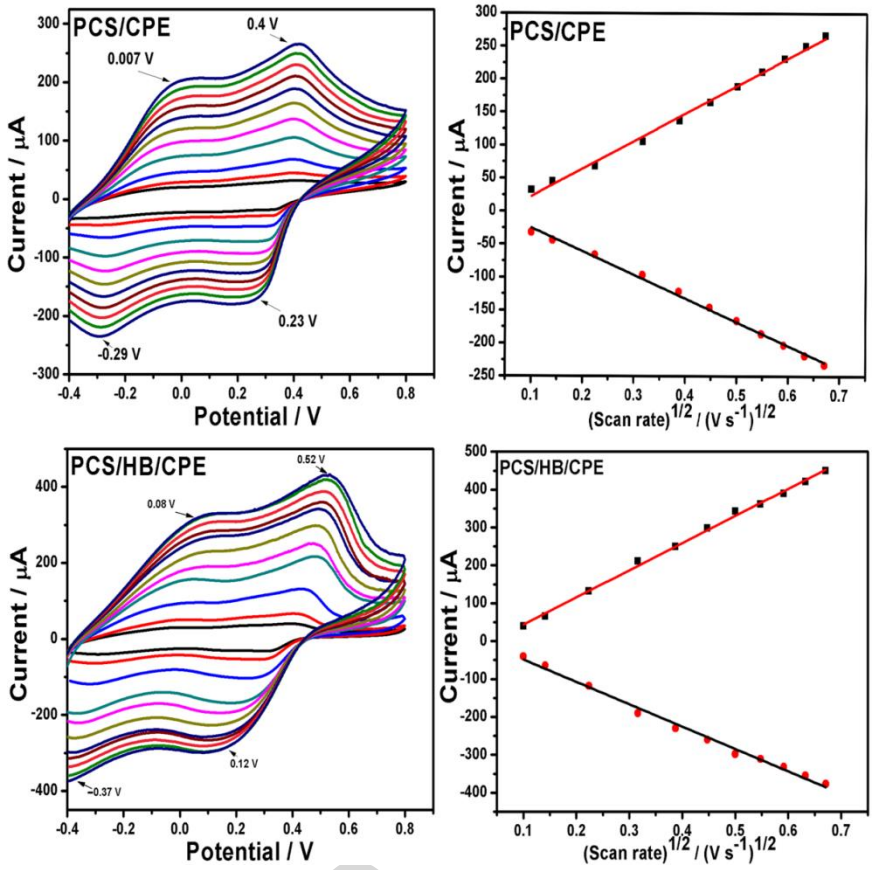

Fig. 3 Cyclic voltammograms of PCS/CPE, PCS/HB/CPE, recorded at different scan rate (from 20 to 450 mV/s, in 0.1 M PBS-3) and Randles–Sevcik plots

3.3.The Sensor response to Hydrogen Peroxide

The electrocatalytic activities of the unmodified (bare CPE) and modified electrodes (PCS/CPE, PCS/HB/CPE) were investigated by the cyclic voltammetric response of 1.0 mM H_2O_2, in 0.1 M PBS-7 solutions, scan rate 20mV/s, potential range from -400 mV to +800 mV (vs. Ag/AgCl) (fig. 4 A). Bare carbon paste electrode did not show any significant signal, however, a pair of well-defined redox couple (anodic peak at 200 mV and cathodic peak at -100 mV, associated with oxidation and reduction of the emeraldine form of PANI) was observed for modified carbon paste electrode.

With the addition of hydrogen peroxide, the cathodic peak shifted toward more negative potential (-123.54 mV to -173.01 mV) and anodic peak shifted toward more positive potential (E_a 178.96 mV to 205 mV). In addition, a significant rise in cathodic current (I_c: -50.54 µA to -66.07 µA and I_a: 41.94 µA to 53.72 µA), which attributed reduction of Hydrogen peroxide at lower potentials as:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

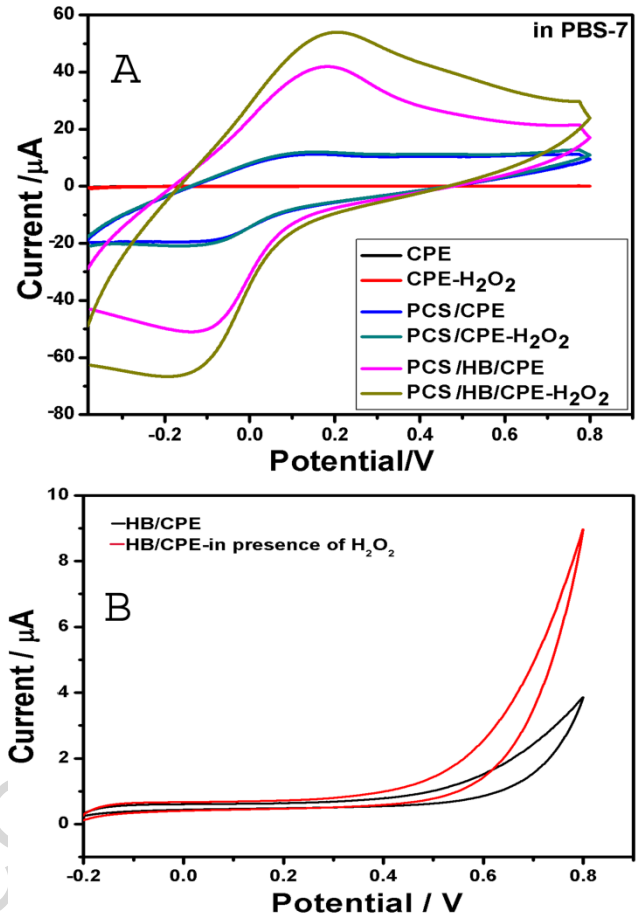

Fig. 4 (A) Cyclic Voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE in 0.1 M PBS-7 with 0.1 mM H_2O_2 solution at a scan rate of 20 mV/s, (B) Cyclic Voltammetry of HB/CPE in 0.1 M PBS-7 with 0.1 mM H_2O_2 solution at scan rate of 20 mV/s

It is very interesting to note that HB modified carbon paste electrode does not show any reduction peak, which indicates that pure HB-CPE could not reduce H_2O_2 efficiently, attributed to the hidden electro-active center (fig. 4B). The observed increment in the anodic current (3.8 µA to 8.9 µA at potential 700 mV vs. Ag/AgCl reference electrode) was due to electrocatalytic oxidation of H_2O_2, according to the equation;

$$\mathbf{H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-}$$

The peak current of cyclic voltammograms increases with the successive addition of hydrogen peroxide 10-200 µL in the electrolyte solution (fig. 5A). A linear calibration plot was obtained using cathodic peak current as a function of peroxide concentration in the electrolyte solution (fig. 5B). The cathodic peak shows more increment in current than anodic, attributed to dominating reduction process). The current response stabilizes to the maximum limit in spite of the further addition of peroxide, attributed to the saturation of the electrode. The electrode showed linear range (0.1 - 5 mM), limit of detection (0.032 mM) (S/N=3), correlation coefficient (R^2 = 0.9975), sensitivity (76.43 µA/mM cm^2) and response time (2s).

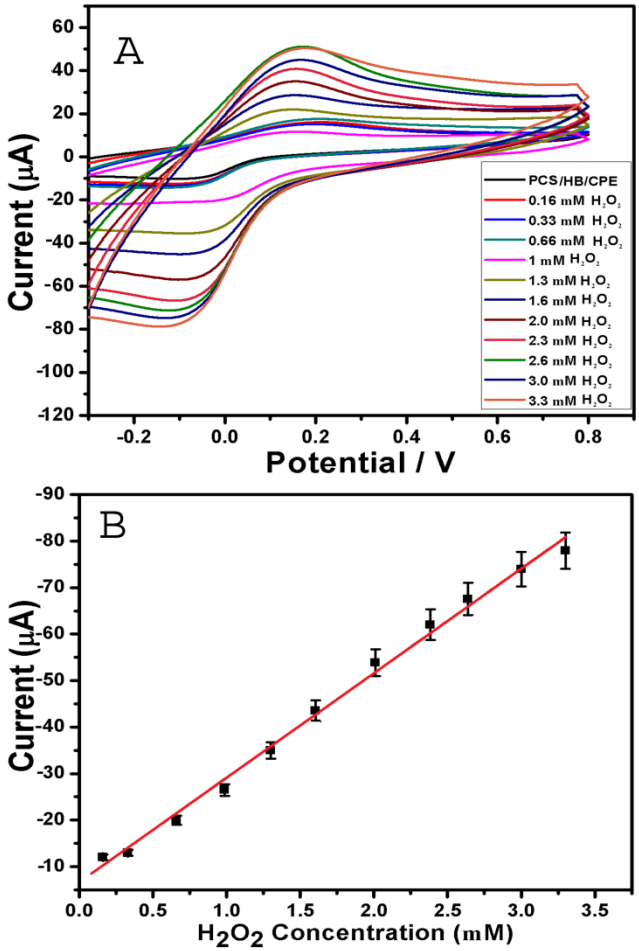

Fig. 5 (A) Cyclic voltammograms of the PCS/HB/CPE modified electrode in the presence of different concentration of H_2O_2, in 0.1M PBS-7 at a scan rate of 20 mV/s, (B) Calibration plot (cathodic current versus concentration of H_2O_2)

3.4. Effect of pH

Generally, fabrication and operation of biosensors required the optimization of solution pH in order to maintain the functionality and structural integrity of biocatalyst. On changing the pH, the amino acids at the active site could ionize, which result in catalytic poising. Polyaniline exhibits reversible redox behavior in strongly acidic media due to the large concentrations and high mobility of the counter ions. The loss in electroactivity of PANI in the solution of higher pH associated with leaching of anions and protons. The cyclic voltammograms of PCS/HB-CPE were recorded in the PBS

solutions of different pH (1, 3, 5 and 7) at scan rate 50 mV/s (c.f. S2). Two redox couples were observed, which are associated with proton and anion doping/dedoping. The anions adsorbed on the positively charged PANI particles. The response of hydrogen peroxide also varies with pH of the solution.

3.5. Chronoamperometric studies

Chronoamperometric experiments were performed, by applying a constant step up the potential for a defined period and current monitored with respect to time. The current of the Cottrell plot is a measurement of the rate of electrolysis at the electrode surface. Under the control of diffusion condition, the slope indicates the diffusion constant for forward and reverse step. Chronoamperometry of CPE, PCS-4/CPE, and PCS/HB/CPE was obtained, potential step; -0.4 to +0.4 V vs. Ag/AgCl, time interval 10 s, in PBS 7 solutions contain potassium ferricyanide as a redox marker (fig. 6a). Higher redox current for PCS/HB/CPE than bare CPE and PCS/CPE indicates the compatible nature HB and PCS indicate better charge transfer at interfaces, support CV analysis. In the presence of 0.1 mM hydrogen peroxide, there is a significant increment of current, which indicates that PCS in combination with HB is a good sensing material for the detection of hydrogen peroxide (fig. 6b). Successive additions of H_2O_2 result in a continuous rise in the current response (c.f. S3).

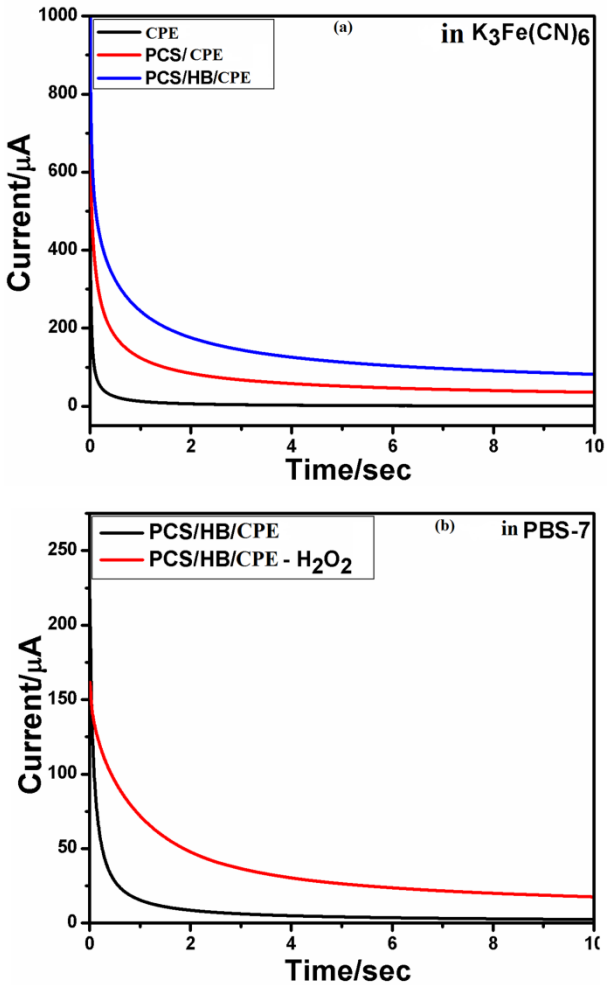

Fig. 6 Chronoamperometry of (a) bare CPE, PCS/CPE, PCS/HB/CPE, (Supporting electrolyte: PBS-7 and 0.1 mM solution of $K_3Fe(CN)_6$ as redox marker), (b) PCS/HB/CPE and PCS/HB/CPE-H_2O_2 (supporting electrolyte: 0.1 M PBS-7 solution)

3.6. Chronocoulometric studies

Chronocoulometry is a controlled-potential electrochemical technique to measure the charge transferred with respect to time (t). Chronocoulometric curves for CPE, PCS/CPE, PCS/HB/CPE was obtained in the presence of potassium ferricyanide (as redox marker) and H_2O_2, using PBS -7 as an electrolyte, the potential range -400 mV to +400 mV vs. Ag/AgCl, step width was 50 s (c.f. S4). PCS/HB/CPE showed

better charge transfer than PCS/CPE, attributed to an effective communication between PCS nanoparticles and the hidden redox center of HB.

3.7. Electrochemical Impedance Spectroscopy (EIS)

It is a non-destructive and very sensitive technique to determine the intrinsic conductivity and interface properties of the modified electrode. The impedance is a sum of the real impedance (Z' (Ω)) and imaginary impedance (Z"(Ω)), corresponds to the resistance and capacitance of the cell. The Nyquist plot of the EIS includes a semi-circular portion and a linear portion. The linear segment at lower frequencies shows a controlled diffusion process; the semicircle part at higher frequencies corresponds to the electron transfer limited process or the charge transfer resistance (Rct) (attributable to the process at the polymer–electrolyte interface). Electrochemical activities were recorded in the open-circuit potential approximate (200 mV) with 1.0 mM $K_3Fe(CN)_6$ (fig. 7a), and with H_2O_2 in 0.1M PBS-7 (fig. 7 b,c). Bare CPE showed higher surface resistance than modified electrode; the developed composite material and HB are compatible with each other, so we observed a facilitated charge transfer in the presence of hydrogen peroxide. Thus, conductive of the electrodes is as follows - CPE < PCS/CPE < PCS/HB/CPE.

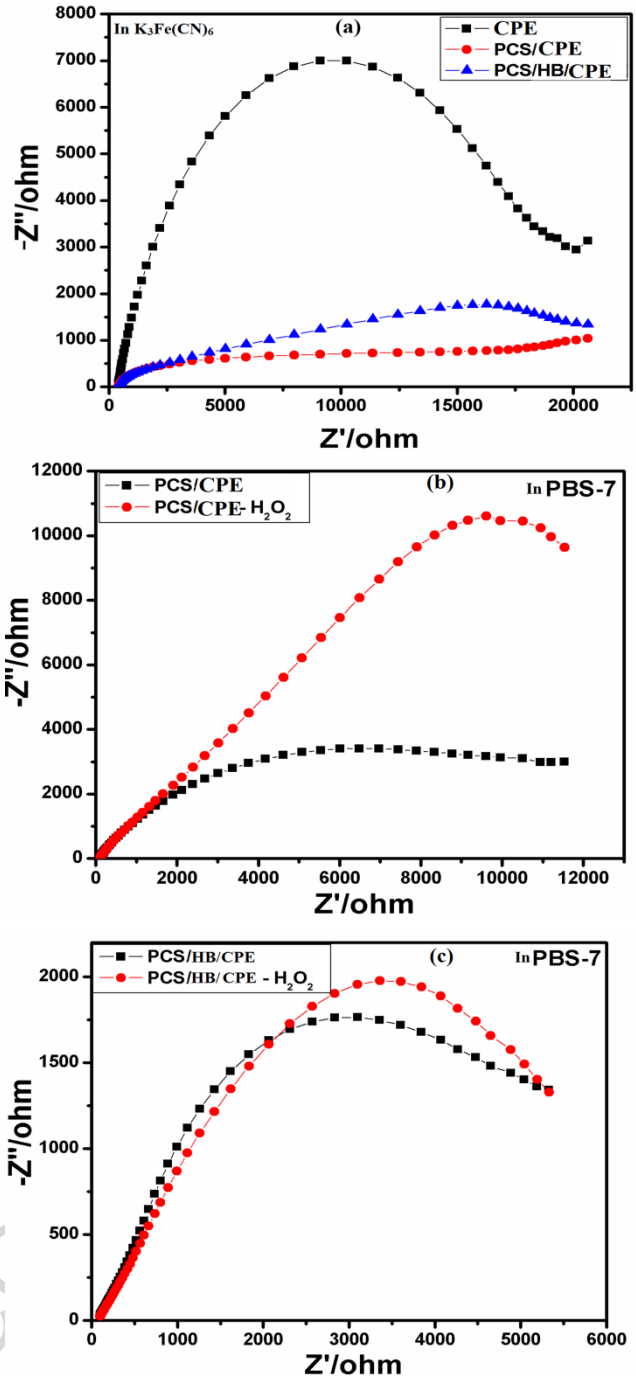

Fig. 7 Electrochemical impedance spectra in 0.1 M PBS-7 (a) bare CPE, PCS/CPE, PCS/HB/CPE with $K_3Fe(CN)_6$(b) PCS/CPE with 0.32 mM H_2O_2, (c) PCS/HB/CPE with 0.32 mM H_2O_2

3.8. Response of some other analytes and interference study

Selectivity of the sensor was estimated by CV in the presence of other analytes (ascorbic acid, catechol, glucose, l tyrosine, gallic acid, l dopa and glycine). The response was comparatively analyzed in term of percentage increase of cathodic peak current with respect to blank (fig.8). We observed the highest current response of hydrogen peroxide (225% increment with respect to blank). Interference study indicated that no significant impact on the response of hydrogen peroxide (c.f. S5).

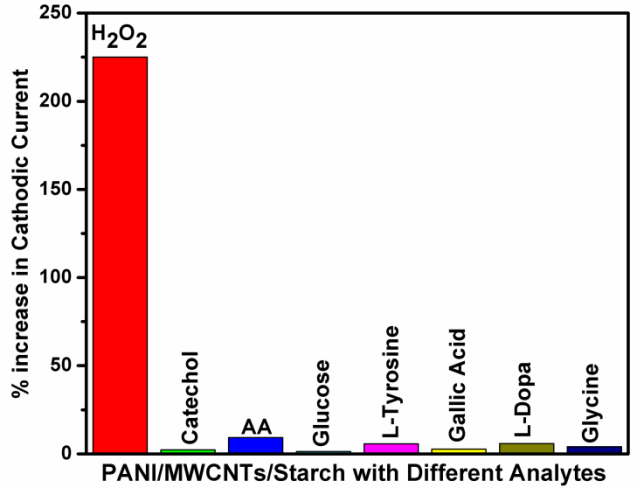

Fig. 8 Percentage increase in cathodic current response with respect to blank for different analytes on [PANI/MWCNTs/Starch]-HB modified carbon paste electrode

3.9. Application for PCS/HB/Gox for Glucose sensing

We fabricated a carbon paste electrode by using PCS, HB and Glucose oxidase (GOD) to detect glucose. Enzymatic oxidation of glucose release hydrogen peroxide. We comparatively study the electrochemical sensing of glucose by using PCS/CPE, PCS/HB/CPE and PCS/HB/GOD/CPE in PBS-7(Fig. 9). The calibration curves were plotted by measuring cathodic current with respect to the concentration of glucose (c.f.

S6). We observed that PCS/HB/GOD/CPE showed better than others in terms of higher peak current and more reversible electrode kinetics.

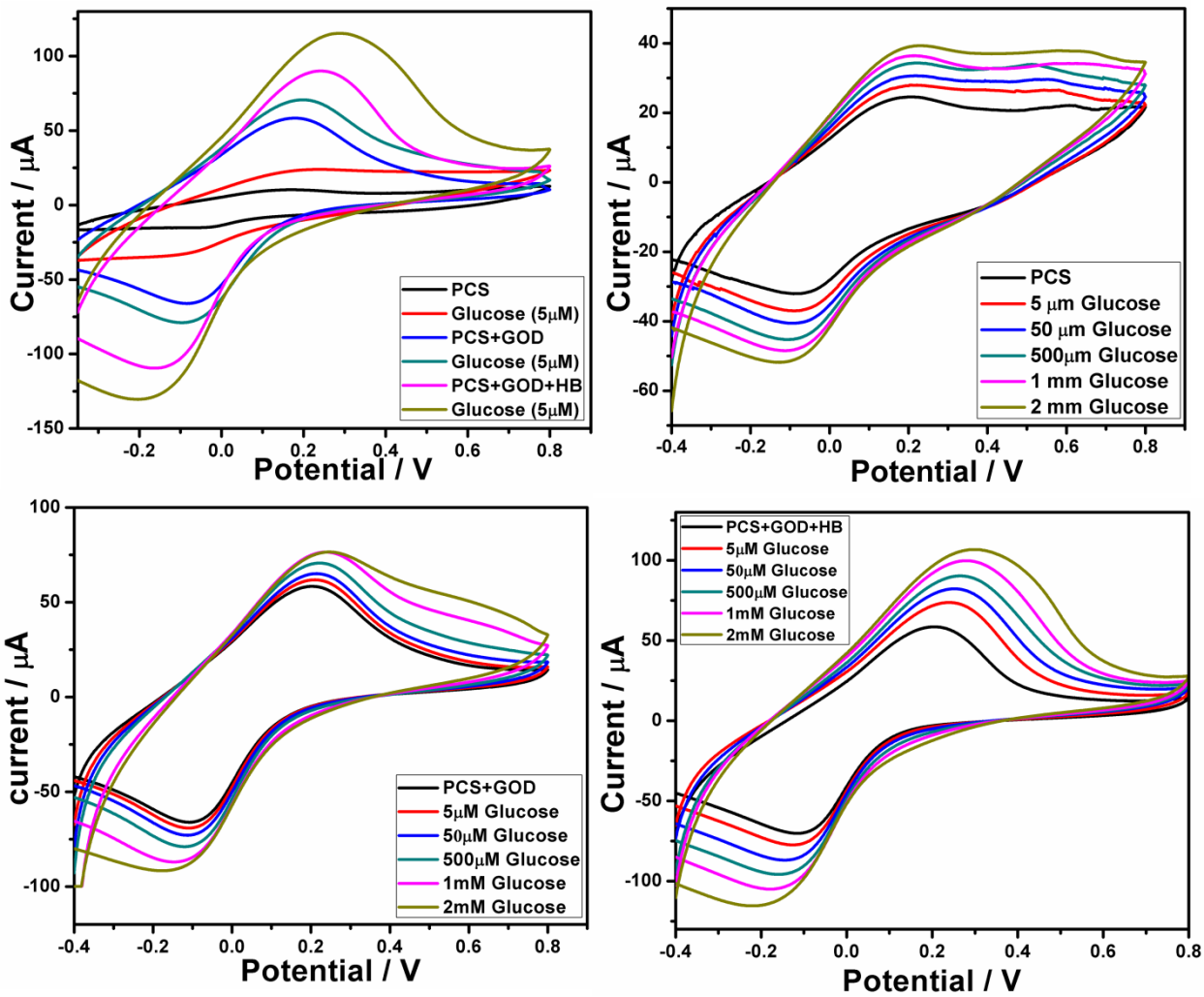

Fig. 9 CV response of Glucose on PCS, PCS-HB, PCS/HB/God modified CPE

4. Conclusions

We have successfully fabricated H_2O_2 electrochemical sensor by using PCS-HB-CPE. The HB and PCS are compatible with each other; in combination they promote the reduction of hydrogen peroxide. PCS facilitate the charge transfer during the electrocatalytic reduction of hydrogen peroxide, confirm from the rise in cathodic current with the successive addition of analyte in the test solution. ESI studies

indicated that the proposed biosensor system has lower charge transfer interfacial resistance than the bare electrode. The characteristics of the proposed biosensor; linear range from 0.1-5mM, correlation coefficient R^2 = 0.9975, lower detection limit (0.032mM), higher sensitivity 76.43 μA/mM cm^{-2} and fast response time (2s). PCS/HB/CPE based biosensor has good operational/storage stability and selective response H_2O_2. We have successfully detected glucose by using glucose oxidase along with PCS and HB modified carbon paste electrode. Work is under process to develop water-based electroactive ink formulation for low-cost sensor strips.

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Conflict of interest: The authors declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Schematic diagram of functioning of peroxide biosensor, and molecular structures of MWCNTs, starch, polyaniline, and hemoglobin

Fig. 2 Cyclic voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE, at scan rate of 20 mV/s (A) in 0.1M PBS (pH 7.0) (B) in presence of Potassium Ferricyanide solution in 0.1 M PBS-7

Fig. 3 Cyclic voltammograms and Randles–Sevcik plots of PCS/CPE, PCS/HB/CPE, recorded at different scan rate (from 20 to 450 mV/s) in 0.1 M PBS-3

Fig. 4 (A) Cyclic Voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE in 0.1 M PBS-7 with H_2O_2 solution at scan rate of 20 mV/s, (B) Cyclic Voltammetry of HB/CPE in 0.1 M PBS-7 with 0.1 mM H_2O_2 solution at scan rate of 20 mV/s

Fig. 5 (A) Cyclic voltammograms of the PCS/HB/CPE modified electrode in the presence of different concentration of H_2O_2, in 0.1M PBS-7 at a scan rate of 20 mV/s; (B) Calibration plot (cathodic current versus concentration of Hydrogen Peroxide)

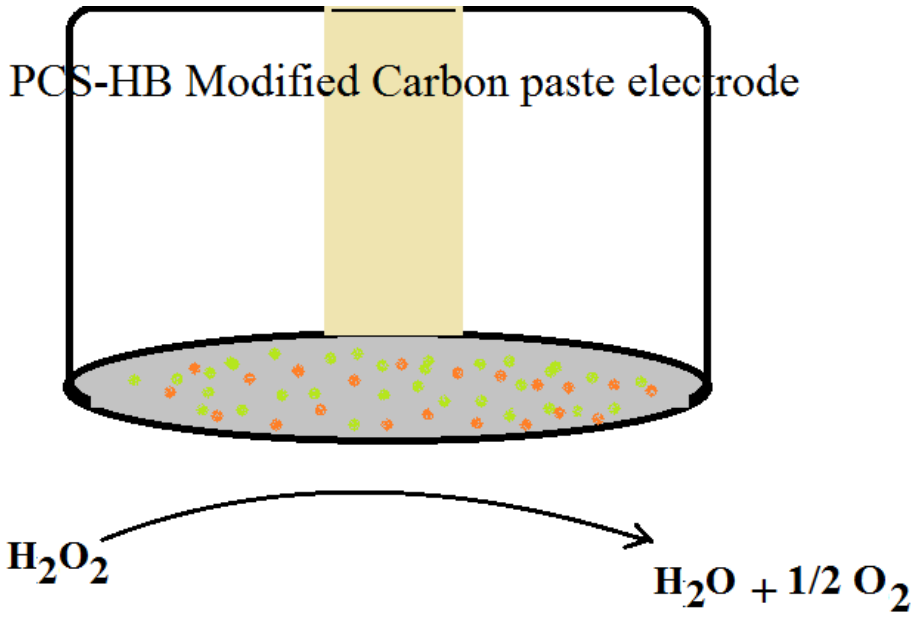
Fig. 6 Chronoamperometry of (a) bare CPE, PCS/CPE, PCS/HB/CPE, (Supporting electrolyte: PBS-7 and 0.1 mM solution of $K_3Fe(CN)_6$ as redox marker), (b) PCS/HB/CPE and PCS/HB/CPE-H_2O_2 (supporting electrolyte: 0.1 M PBS-7 solution)

Fig. 7 Electrochemical impedance spectra in 0.1 M PBS-7 (a) bare CPE, PCS/CPE, PCS/HB/CPE with $K_3Fe(CN)_6$, (b) PCS/CPE with 0.32 mM H_2O_2, (c) PCS/HB/CPE with 0.32 mM H_2O_2

Fig. 8 Percentage increase in cathodic current response with respect to blank for different analytes on [PANI/MWCNTs/Starch]-HB modified carbon paste electrode

Fig. 9 CV response of Glucose on PCS, PCS-HB, PCS/HB/God modified CPE

Graphical abstract



In this work, we proposed a novel nonenzymatic hydrogen peroxide biosensor based on PANI/MWCNTs/Starch composite material (PCS), using haemoglobin (HB) as a low cost biocatalyst. The developed biosensor showed a linear relationship between cathodic current and concentration of hydrogen peroxide (0.1 mM - 5 mM, $R^2 = 0.9975$), limit of detection (0.032 mM), sensitivity (76.43 µA/mM cm^2) and long term storage

Research highlight

1. The novel composite material PANI/MWCNTs/Starch is promising electrode material for biosensor applications.
2. Morphological studies by SEM, HRSEM, TEM
3. Elemental analysis by EDAX
4. Electrochemical studies: CV, Chronoamperometry, Chrnocoulometry, and ESI
5. The system exhibited following characteristics; low cost, good dispersion ability, conductive, electroactive, multifunctional, integrated nano morphology, could communicate with biocatalyst, biocompatible and environment friendly, and redox active, long term stability, easy process ability.
6. Hydrogen peroxide sensor is developed as a model system.
7. Hemoglobin used as low cost biocatalyst for electrocatalytic reduction of hydrogen peroxide.
8. This material could be easily integrated with other enzyme system, and used as a common platform to develop other biosensors.
9. We have incorporate a little amount of glucose oxidase with “PCS+HB” and successfully used to detect glucose.