The multi-component material systems have manifold interactions and synergistic properties. We have prepared a novel nanocomposite material by insitu-chemical polymerization of aniline in the aqueous suspension of starch and MWCNTs. It has high surface area, good electro-activity, conductivity, stable aqueous dispersion, bio-compatibility, both hydrophilic and hydrophobic regions, multifunctionality and porous nano-turmeric shape morphology. In this chapter, we propose a novel nonenzymatic hydrogen peroxide biosensor based on PANI/MWCNTs/Starch nano-composite material (designated as PCS) and hemoglobin (HB) modified carbon paste electrode. Electrochemical studies revealed that PCS + HB modified carbon paste electrode (CPE) showed better charge transfer mechanism at the electrode interface (potassium ferricyanide used as an electroactive marker). Interestingly, compatibility of PCS and HB facilitates the electrocatalytic reduction of hydrogen peroxide, which results in a significant rise in the cathodic current. On the other hand, while modification of the carbon paste electrodes was done by hemoglobin and PCS individually (HB-CPE and PCS-CPE), no significant increment in cathodic current response was detected. The results indicate that nano particles of PCS effectively communicate with HB molecules in the carbon paste. A mixture of PCS + HB is a cost-effective electrode material for sensitive and selective detection of hydrogen peroxide. The developed biosensor showed a linear range (0.1 mM - 5 mM, $R^2 = 0.9975$) with limit of detection (0.032 mM), sensitivity (76.43 $\mu$A/mM cm$^2$) and long-term storage and stability. This material system along with glucose oxidase was applied to detect Glucose.

## 6.1 Introduction:

Biosensor is a bioanalytical device which consists of suitable biological sensing elements integrated with transducer and electronic amplifier. In recent years, development of functional nanocomposite materials for biosensor system has been an interesting area of research due to their small size, large surface-to-volume ratio, high surface activity, selective interaction and excellent catalytic activity. Enzymatic bio-sensors are one of the most explored electro-analytical system because enzymes have an inherent selective catalytic reaction with the substrate and are commercially available. Enzymes are expensive bio-molecules and there is a need of maintaining their structural integrity, catalytic activity and an effective relay of the electrical signal at the electrode surface. Proper attention has to be given on the immobilization because the activity and structure of enzymes are very sensitive to chemical and physical parameters, such as temperature, pH and humidity. Poisoning of enzyme molecules degrades the biosensor performance. The stability of enzymes on the transducer surface is a great challenge in front of sensor technologists because the results of enzymatic biosensors suffer from poor reproducibility under environmental fluctuations. Immobilization techniques are complicated and multi-step events. Efforts are going on to develop nonenzymatic sensor systems by using stable proteins and other nano-catalysts. For electrochemical sensor applications, the electrode material should have good transport of charge and mass, support electro-activity, bio-compatibility, good dispersion and environmental stability (Holzinger et al. 2014; Kurbanoglu et al. 2017).

Determination of hydrogen peroxide ($H_2O_2$) has found much importance in many fields, such as environmental, food science, pharmaceutical chemistry, biochemistry, chemical, industrial, medical, cosmetics and biological processes. A reliable and economical method for the determination of $H_2O_2$ is of great significance. It is used for disinfection, odor control, oxygenation, cyanide oxidation etc. In the biological system, controlled production of hydrogen peroxide has an impact on cell signaling and kills pathogens. In the diseased conditions, mitochondrial respiration and many enzymatic reactions lead to uncontrolled production of hydrogen peroxide. Hence, it's efficient detection could be used as a tool to measure the concentration of some other important biomarkers. Many advance methods have been used for qualitative and quantitative analysis of peroxides e.g. high performance liquid chromatography, spectroscopy, chemiluminescence, titrimetry, iodometry and electroanalytical techniques. A variety of catalysts have been used for electrochemical detection of peroxides such as HRP, Catlase, Myoglobin, Hemoglobin, Prussian blue and others (Tiwari et al. 2010; Boujakhrout et al. 2016; Sies et al. 2017; Chen et al. 2012).

Among various redox proteins, heme-proteins are one of the most widely studied biocatalysts. Hemoglobin (HB), the 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 of pyrrole molecules cyclically linked together by methine bridges) with the iron ion bound in the center - known as a porphyrin). The two alpha chains, each with 141 amino acids, and two beta chains, each with 146 amino acids. The center iron ion coordinates with the four nitrogen atoms (lie in one plane). The mammalian hemoglobin molecule can bind up to four oxygen molecules and iron is the active binding site. When oxygen is not bound, a water molecule fills the site through weak bonds. Even though carbon dioxide is also carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is bound to the protein chains of the structure. HB is a cost effective biocatalyst for the selective reduction of $H_2O_2$ and is easily available in the market and has better operation/storage stability. Thus, HB could be used for commercial fabrication of peroxide bio-sensors. However, the electron transfer reactivity of HB has a physiological disadvantage as the electroactive center is hidden within the protein sheath and it renders ‘signal capture’ during the biological recognition event at electrode surface 'a challenge'. A variety of redox mediators, nano particles and substrates have been used successfully for electrochemical detection of $H_2O_2$ (Sun et al. 2014; Sun et al. 2016; Matysiak et al. 2015; Oja et al. 2016; Wang et al. 2015).

The electroactivity of the PANI greatly depends upon the degree of oxidation and extent of protonation. Conductivity and electro-activity of the PANI samples gradually decrease in the solution of higher pH. Nano fillers and bulky anionic dopants could suppress the deprotonation process or provide a local acidic environment within the PANI matrix. A hybrid material system has superior properties and it is a good approach to overcome the individual deficiency of materials (Dhand et al. 2011; Park et al. 2015; Nambiar et al. 2011; Shukla et al. 2015; Tiwari et al. 2012; Song et al. 2013; Tiwari et al. 2009).

Starch $(C_6H_{10}O_5)_n$ is an abundantly available polysaccharide, produced by green plants as an energy storage and is used in human diets. It comprises glucose monomers joined together by α 1,4 linkages, contains 20 to 25% amylose and 75 to 80% amylopectin by weight. It is white, tasteless and odorless amorphous micro-granular powder. Polysaccharides are insulators and non electroactive but they provide better solubility and biocompatible environment for the immobilized bio-catalysts particularly enzymes (small sugar molecules and poly-ol molecules support to maintain stability of enzymes) (Lu et al. 2009; Homaei et al. 2013; Zhang et al. 2014). Polyaniline–polysaccharide composite systems have higher resistance than pristine PANI because insulating starch hinders the inter-granular charge transfer. The idea of reinforcing a conducting nano-filler could improve the electrical conductivity as well as the mechanical properties of the composite material.

Carbon nanotubes have emerged as a very interesting nano-material, and find applications in many fields due to high conductivity, chemical stability, good strength with phenomenal unique structure and very high surface-to-volume ratio. A trace amount of nano fillers could drastically modify the characteristics of the composite material, particularly surface area, strength, electrical and electrocatalytic properties (Barsan et al. 2015; Volder et al. 2013; Tiwari et al. 2012).

PANI-polysaccharide composite systems are promising electrode material for sensor applications. In chapter 4, we have studied the important characteristics of a novel ternary composite material based on PANI, MWCNTs and Starch. This ternary composite material is better than the binary system in term of good electro-activity, conductivity, multifunctionality and porous nano turmeric shape architecture. It forms a stable dispersion in aqueous medium due to the presence of hydrophilic/hydrophobic regions, porous architecture and numerous hydroxyl functional groups. Starch improves the electro-activity by compensating positive charge of PANI, whereas MWCNTs as a template and nano-filler, which improve the bulk conductivity of the material, passage to transfer redox current through inter-grains and increases the surface area of composite material (Gautam et al. 2017; Gautam et al. 2016).

Electrochemical application of [PANI/MWCNTs/Starch] composite for biosensors is favoured by its biocompatibility, electroactivity and porosity. It is a low-cost elecrocatalytic material is of immense importance to fabricate biosensor. HB was used as biocatalyst and the result shows that PCS with HB is effective to promote the electrocatalytic reduction of $H_2O_2$. Since hydrogen peroxide is generated in many enzymatic redox reactions, its detection could be used to estimate other analytes using bi-enzyme electrode system. We have applied this system to detect glucose, using glucose oxidase as a biocatalyst.

## 6.2 Materials and Methods:

### 6.2.1 Reagents and Materials:

The chemicals and reagents required for this research work were procured from the local vendors: 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] were purified by boiling with concentrated HCl. Phosphate buffer solution (PBS, 0.1 M) was prepared with stock solutions of $Na_2HPO_4$ and $NaH_2PO_4$. The 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 before its use. Starch was used without any further purification and all the solutions were prepared using double distilled water.

### 6.2.2 Instrumental Analysis:

Electrochemical Analyzer/Workstation model 600D Series, CH instruments, USA was used to carry out different electrochemical analyses such as cyclic voltammetry, chronoamperometry, chronocolumetry and electrochemical impedance spectroscopy (EIS). The three electrode system consisted of 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 Quanta 200 Company- FEI of USA (SEA) PTE Ltd., Singapore at suitable voltages and magnifications. Transmission electron microscopy (TEM) examination was made by using FEI Company Technai 20G2 electron microscope at an accelerating voltage of 200 KV.

### 6.2.3 Preparation of Composite Material:

PANI/MWCNTs/Starch composite (designated as PCS) was prepared by the in-situ chemical oxidative polymerization of aniline in the suspension of starch and MWCNTs at low temperature, extensively discussed in chapter 5 (Gautam et al. 2017; Gautam et al. 2016).

### 6.2.4 Fabrication of Carbon Paste Electrodes:

Fabrication of carbon paste electrode is advantageous in terms of low background current, renewable or disposable electrochemical interface, low-cost fabrication, simple preparation, specificity and stability (Vytras et al. 2009; Auley et al. 2015). Carbon paste electrode was prepared by manual mixing of graphite powder (0.1 g), the composite material (0.01 g) and nujol oil (12 µL) in a mortar pestle. A part of the resulting homogeneous paste was packed firmly into a glass capillary. The electric contact was established through a copper wire to the end of the paste in the inner hole of the capillary. The prepared electrode surface of PCS/CPE was polished by smoothening on a clean butter paper. Further, it was used as a working electrode. HB (0.001 g), composite material (0.01 g) and graphite powder (0.1 g) were mixed properly and applied to the tip of the modified capillary electrode. For glucose analysis, 0.001 g glucose oxidase was mixed in the carbon paste.

## 6.3 Results and Discussion:

### 6.3.1 Mechanism of Peroxide Sensing:

A multicomponent system has complex interactions and the individual components synergistically contribute to the property of the final material. Figure 6.1 shows the schematic diagram of the sensor mechanism and molecular structures of individual constituents for ternary nanocomposite material. PCS is a good electroactive nano-composite material. It acts as charge mediator and effectively communicates to the redox center of hemoglobin. HR-SEM and TEM images confirmed that the PCS nano composite has porous nano-turmeric shape structure (c.f. B1). Hemoglobin is a cost effective bio-catalyst to reduce hydrogen peroxide. PCS particles interact with hemoglobin through weak hydrogen bondings. Poly-hydroxyl functionality of starch provides a local bio-compatible environment, which is an ideal supporting host material and maintains the integration of conformation and catalytic-activity of HB molecule. Due to electro-active and conducting nature of PCS, it facilitated the current flow during the reduction of peroxide. MWCNTs were uniformly distributed within the composite as conducting nano-filler and provide an extra passage for charge transfer and prevents current drainage. Although MWCNTs are not electro-active, they certainly improve the material quality in term of good electrical conductivity, electrochemistry and large surface area. We manually mix PCS, HB, and fine graphite powder to fabricate a capillary carbon paste electrode. The cathodic current increases with increasing $H_2O_2$ concentration in the electrolyte, associated with the reduction of peroxide and oxidation of HB. Thus, compatibility of HB with PCS promotes the catalytic reduction of peroxide. The mechanism of electrochemical reaction could be represented as,

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

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

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

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Net reaction is $H_2O_2 + 2e- + 2H^+ \rightarrow 2H_2O$ (4)

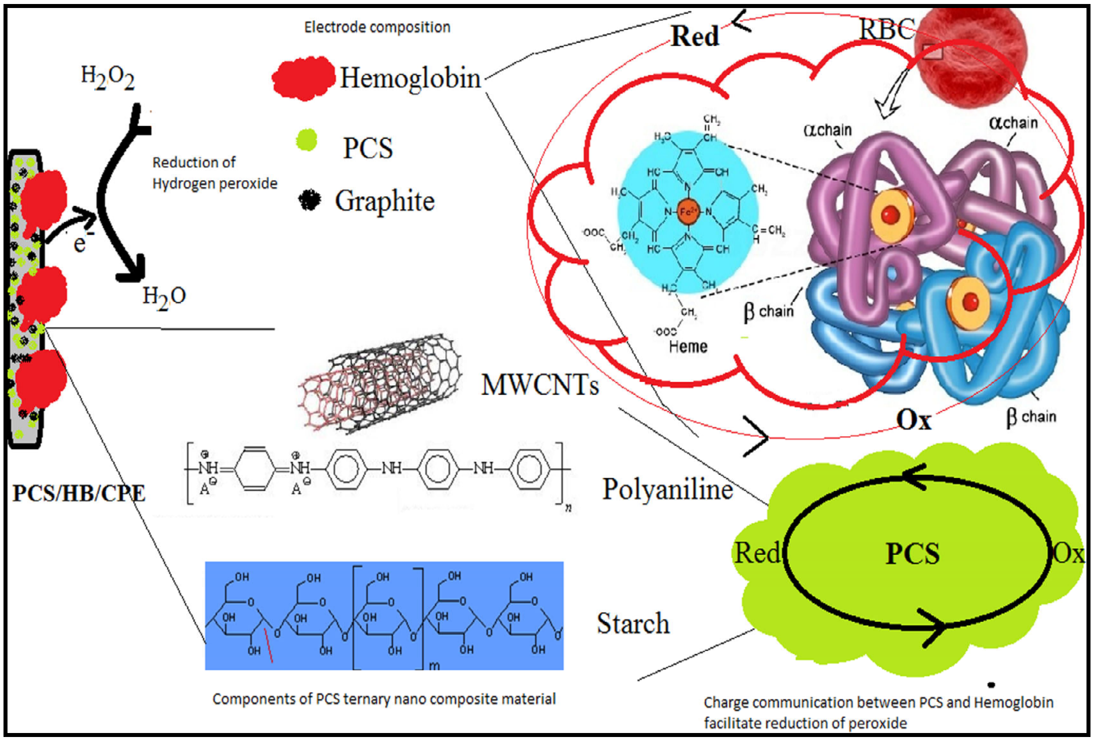

**Fig. 6.1** Schematic diagram of functioning of peroxide biosensor and molecular structures of MWCNTs, starch, PANI and hemoglobin

PCS showed good electrical, electrochemical and surface properties due to interconversion between the oxidation states leucoemeraldine and emeraldine of PANI (starch and MWCNTs are not electro-active materials). The combined effect of MWCNTs and starch on composite material improves electro-activity of PCS than pristine PANI. Electroactivity and conductivity of PANI are closely associated with the degree of oxidation, doping/dedoping level of protons and associated anions, morphology (spatial arrangement of PANI chain) and surface area (Gautam et al. 2016). During the synthesis of PANI in dilute HCl, Chloride ions compensate the positive charge of the chain. In pristine PANI, small size chloride ions easily leach out from the polymer matrix, resulting in a loss in electro-activity at neutral or higher pH. The positive charge on PANI chains get destabilized on de-protonation and ultimately lower the charge transfer capability along the PANI chain. In the strongly acidic solution, three redox couples were observed, which could be associated with doping/dedoping of proton and anions. The voltammograms of ternary and binary systems reveal that PANI/MWCNTs composite material has higher peak current (attributed to conducting filler MWCNTs), whereas PANI/Starch composite showed lower peak current than pristine PANI attributed to the insulation between polyaniline grains due to starch granules, as reported earlier (Prakash et al. 2002).

The cyclic voltammograms of PCS and PCS-HB modified electrode were prepared in 0.1 M PBS-7 with the scan rate of 20 mV/s and potential range -400 mV to +800 mV (fig. 6.2A). Valuable information has been derived from the analysis of CV parameters, viz. 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 ($\Delta E$). We inferred that HB induces fast electrode reaction in a more reversible manner, i.e. it results in lower formal potential and lower peak-peak potential difference. PCS/HB modified electrode showed a steep increase in current rather than a sharp cathodic peak.

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

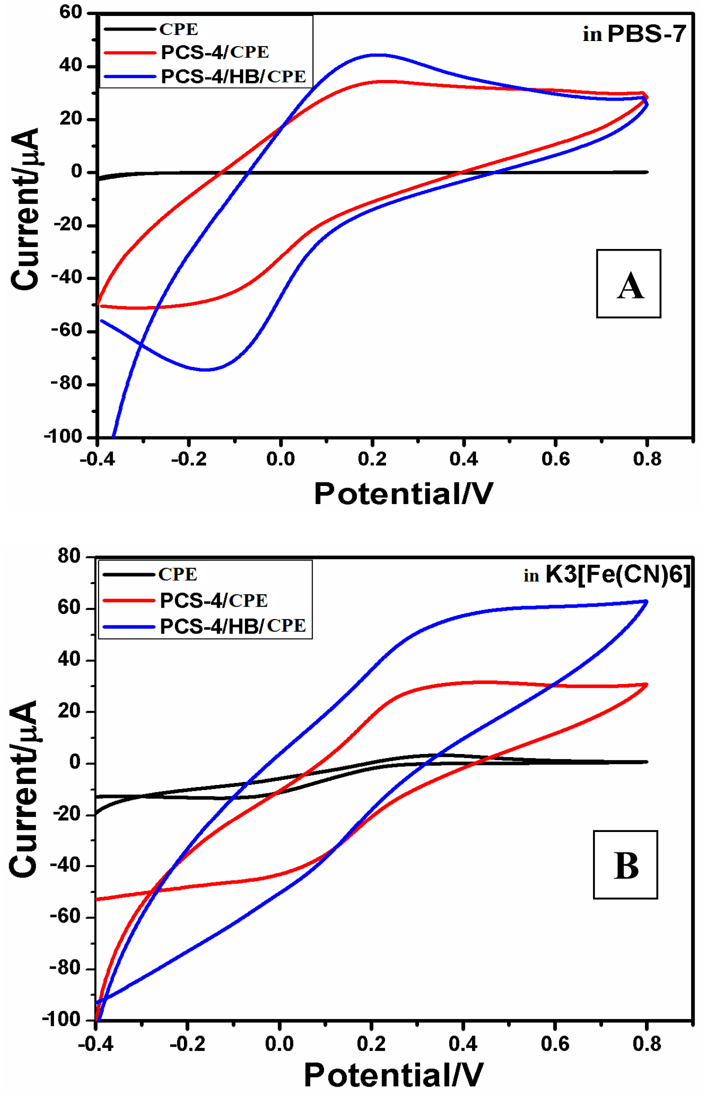

**Fig. 6.2** Cyclic voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE, at scan rate of 20 mV/s (A) in 0.1M PBS (pH 7.0) (B) in presence of Potassium Ferricyanide solution in 0.1M PBS-7

| **Electrodes** | **$I_c$ (µA)** | **$E_c$ (mV)** | **$I_c$ (µA)** | **$E_a$ (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, 10-450 mV/s, in 0.1 M HCl solution. In general, two redox couples were observed 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 controlled electrode process (fig. 6.3). On modified electrode with PCS + HB, the formal potential for first redox couple ($E_1^0$) shifts toward negative potential and ΔE increases. Variation in the cyclic voltammograms strongly suggested the existence of some kind of communication among composite material and hemoglobin.

| **Electrodes** | $E_1^0$ | **ΔE** | $E_2^0$ | **ΔE** |
|---|---|---|---|---|
| PCS/CPE | -151 mV | 156 | 324 Mv | 362 |
| PCS/HB/CPE | -302 mV | 297 | 325 Mv | 450 |

PCS+HB modified electrode has large potential difference in lower pH solution than the neutral solution. This could be explained on the basis of sluggish electrode kinetics due to deterioration of HB. On comparing CV peaks at 100 mV/s and 450 mV/s, with the increased scan rate the anodic peak shifts towards higher potential and cathodic peak shifted towards lower potential and current rises due to greater diffusion. From these observations we infer that the blend of PCS composite material and HB exhibit better electron transfer reaction than individually modified electrodes.

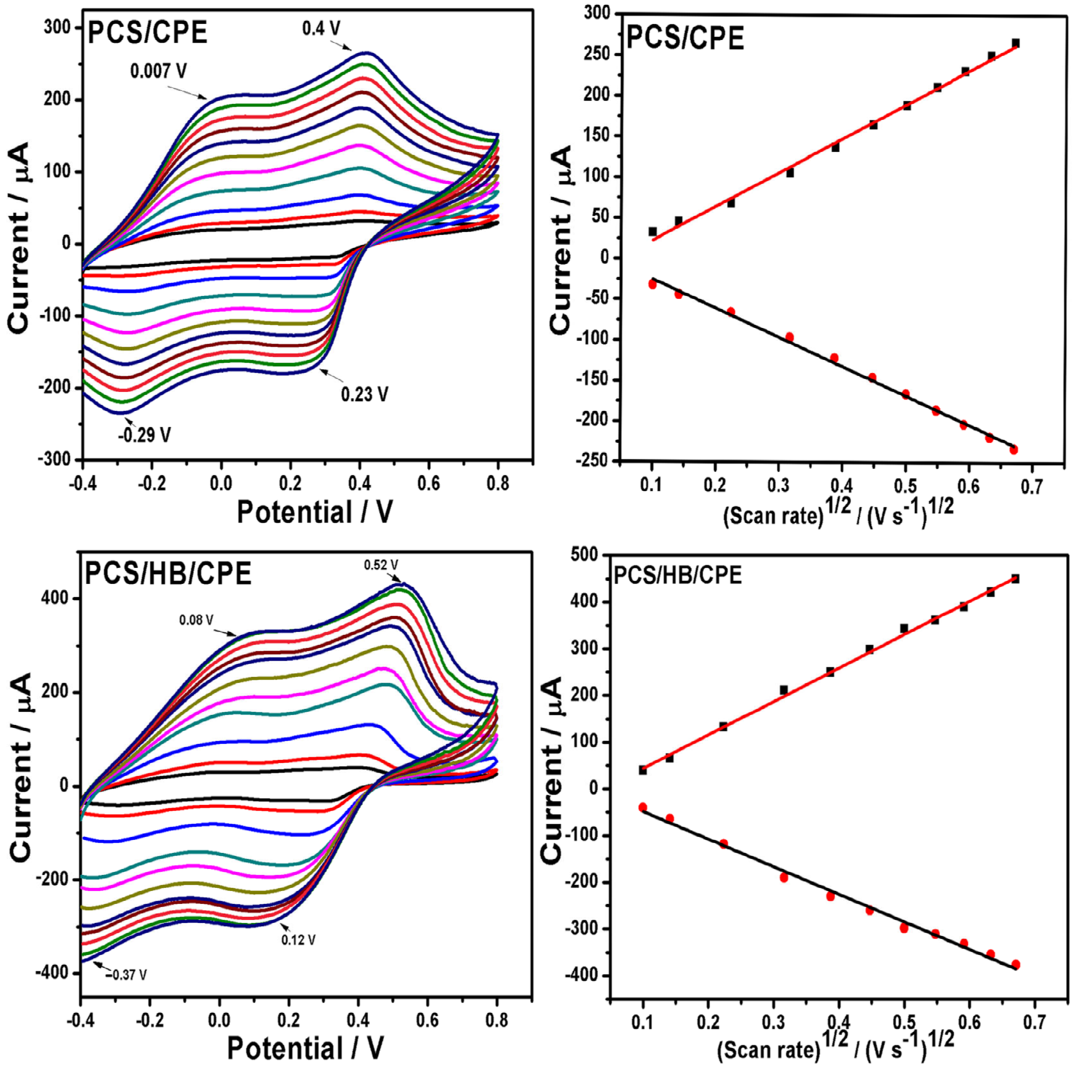

**Fig. 6.3** Cyclic voltammograms and Randles–Sevcik plots of PCS/CPE, PCS/HB/CPE, recorded at different scan rate (from 20 to 450 mV/s) in 0.1 M HCl

| **Electrodes** | **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 |

### 6.3.3 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. 6.4 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 emaraldine form of PANI) was observed for modified carbon paste electrode. With the addition of hydrogen peroxide, the cathodic peak shifted towards more negative potential (-123.54 mV to -173.01 mV) and anodic peak shifted towards more positive potential ($E_a$ 178.96 mV to 205 mV). In addition, a significant rise was observed in cathodic current ($I_c$: -50.54 µA to -66.07 µA and $I_a$: 41.94 µA to 53.72 µA), which is attributed to the reduction of Hydrogen peroxide at lower potentials as:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad (5)$$

It is very interesting to note that HB modified carbon paste electrode does not show any reduction peak, which indicates that pure HB could not reduce $H_2O_2$ efficiently, attributed to the hidden electro-active center (fig. 6.4B). 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:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e- \qquad (6)$$

The peak current of cyclic voltammograms rises with successive addition of hydrogen peroxide 10-200 µL in the electrolyte solution (fig. 6.5A). A calibration plot was obtained with cathodic peak current as a function of peroxide concentration (fig. 6.5B).

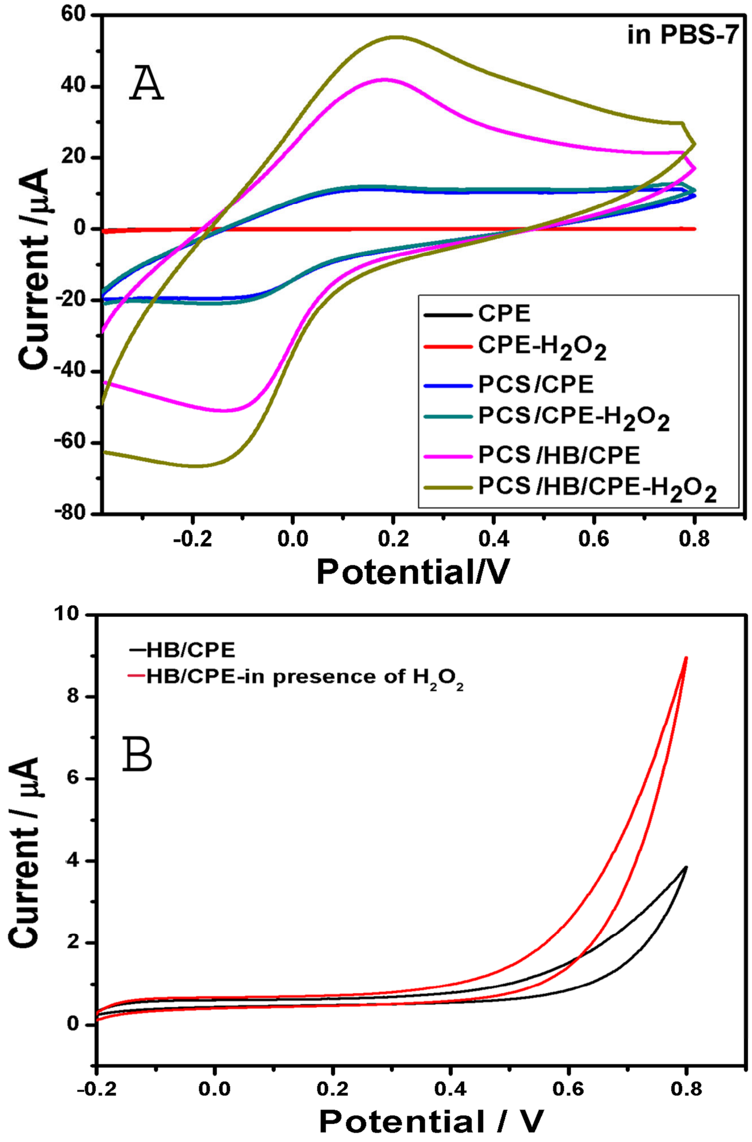

**Fig. 6.4** (A) Cyclic Voltammograms of bare CPE, PCS/CPE, PCS/HB/CPE in 0.1 M PBS-7 with $H_2O_2$ solution at scan rate of 20 mV/s, (B) Cyclic Voltammetry of HB/CPE in 0.1 M PBS-7 with 0.1 mM $H_2O_2$ solution at scan rate of 20 mV/s

The cathodic peak shows more increment in current than anodic (attributed to dominating reduction pocess). 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).

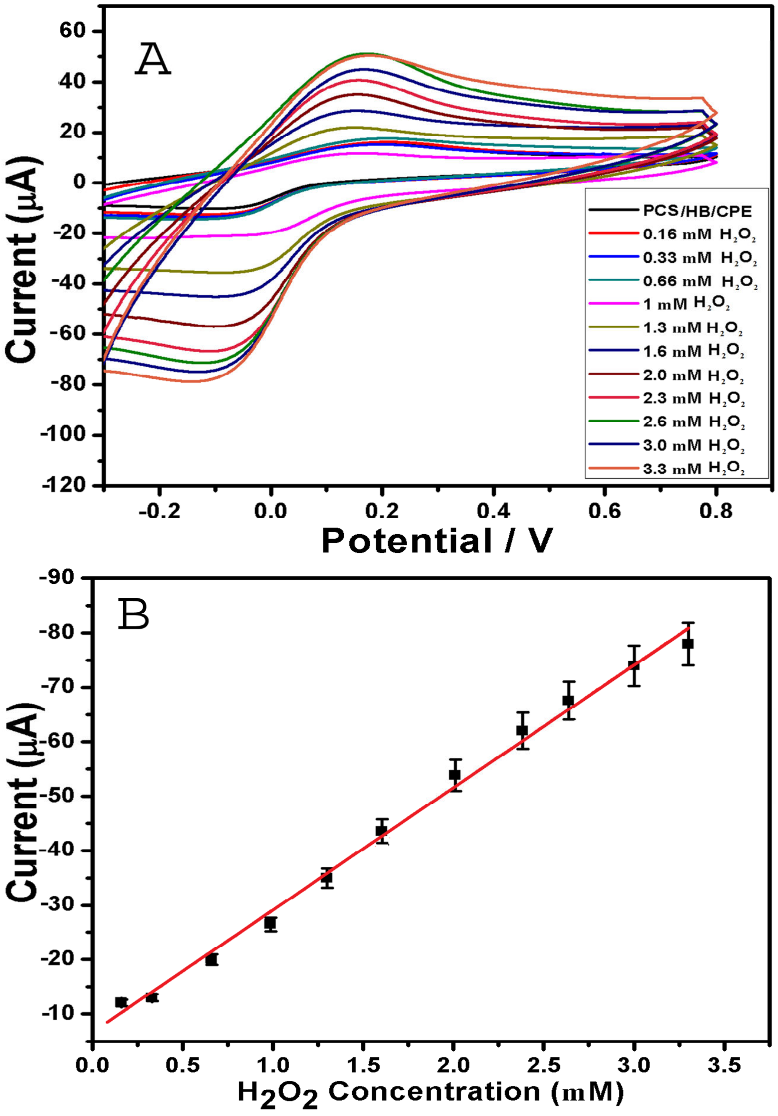

**Fig. 6.5** (A) Cyclic voltammograms of the PCS/HB/CPE modified electrode in the presence of different concentration of $H_2O_2$, in 0.1M PBS-7 at a scan rate of 20 mV/s, (B) Calibration plot (cathodic current versus concentration of Hydrogen Peroxide).

### 6.3.4 Effect of pH:

Generally, fabrication and operation of biosensors requires the optimization of solution pH in order to maintain the functionality and structural integrity of bio-catalyst. The activity of enzyme/bio-molecule changes with the ionization state of the amino acids. 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 is 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. B2).* Two redox couples were observed, which are associated with proton and anion doping/dedoping. The anions get adsorbed on the positively charged PANI particles. The response of hydrogen peroxide also varies with pH of the solution.

### 6.3.5 Chronoamperometric Studies:

Chronoamperometric experiments were performed, by applying a constant step-up in the potential for a defined period and monitoring the current with respect to time. The current of the Cottrell plot is an indicative the rate of electrolysis at the electrode surface. Under diffusion controlled condition, the slope indicates the diffusion constant for forward and reverse step. Chronoamperometry of CPE, PCS-4/CPE, and PCS/HB/CPE was obtained with potential step: -0.4 to +0.4 V vs. Ag/AgCl, time interval 10 sec, in PBS 7 solutions containing potassium ferricyanide as a redox marker (fig. 6.6a). Higher redox current for PCS/HB/CPE than bare CPE and PCS/CPE indicates the compatible nature HB and PCS and there is better charge transfer at interfaces. 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. 6.6b). Successive additions of $H_2O_2$ result in continuous rise in the current response *(c.f. B3).*

### 6.3.6 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 were 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. B4)*. PCS/HB/CPE showed better charge transfer than PCS/CPE, attributed to an effective communication between PCS nano particles and the hidden redox center of HB.

### 6.3.7 Electrochemical Impedance Spectroscopy (EIS):

It is a non-destructive and very sensitive technique to determine the intrinsic conductivity and interface properties of modified electrode. The impedance as 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 diffusion controlled process; the semicircular part at higher frequencies corresponds to the electron transfer limited process or the charge transfer resistance (RCT) (attributed to the process at the polymer–electrolyte interface). Electrochemical activities were recorded in the open-circuit potential (200 mV) with 1.0 mM $K_3Fe(CN)_6$ (fig. 6.7a), and with $H_2O_2$ in 0.1M PBS-7 (fig. 6.7b, 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. The conductivities of the electrodes are as follows - CPE < PCS/CPE < PCS/HB/CPE.

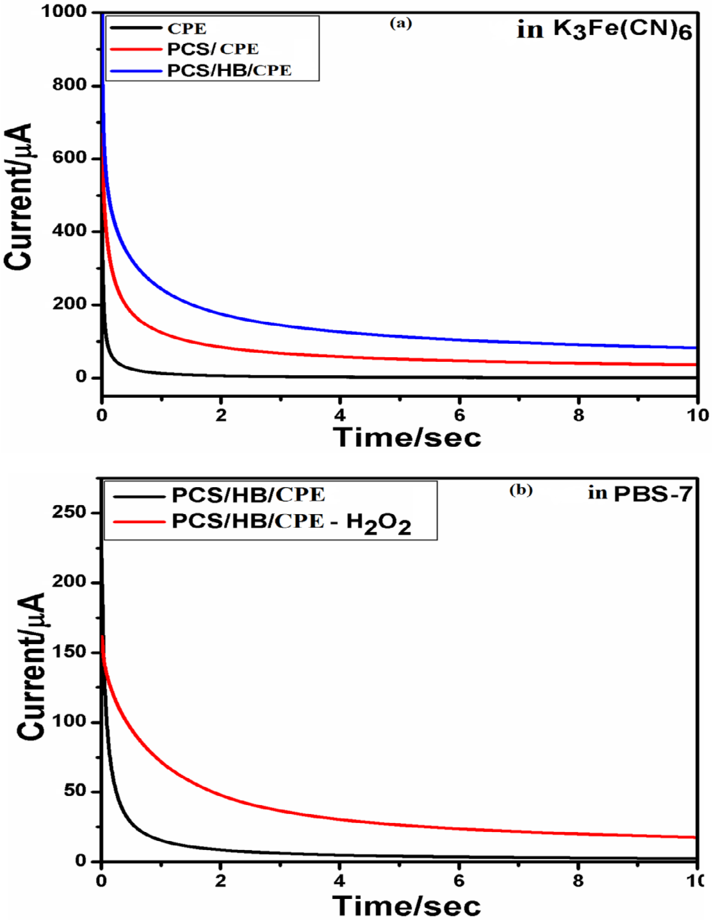

**Fig. 6.6** Chronoamperometry of (a) bare CPE, PCS/CPE, PCS/HB/CPE, (Supporting electrolyte: PBS-7 and 0.1 mM solution of $K_3Fe\ (CN)_6$ as redox marker), (b) PCS/HB/CPE and PCS/HB/CPE-$H_2O_2$ (supporting electrolyte: 0.1 M PBS-7 solution)

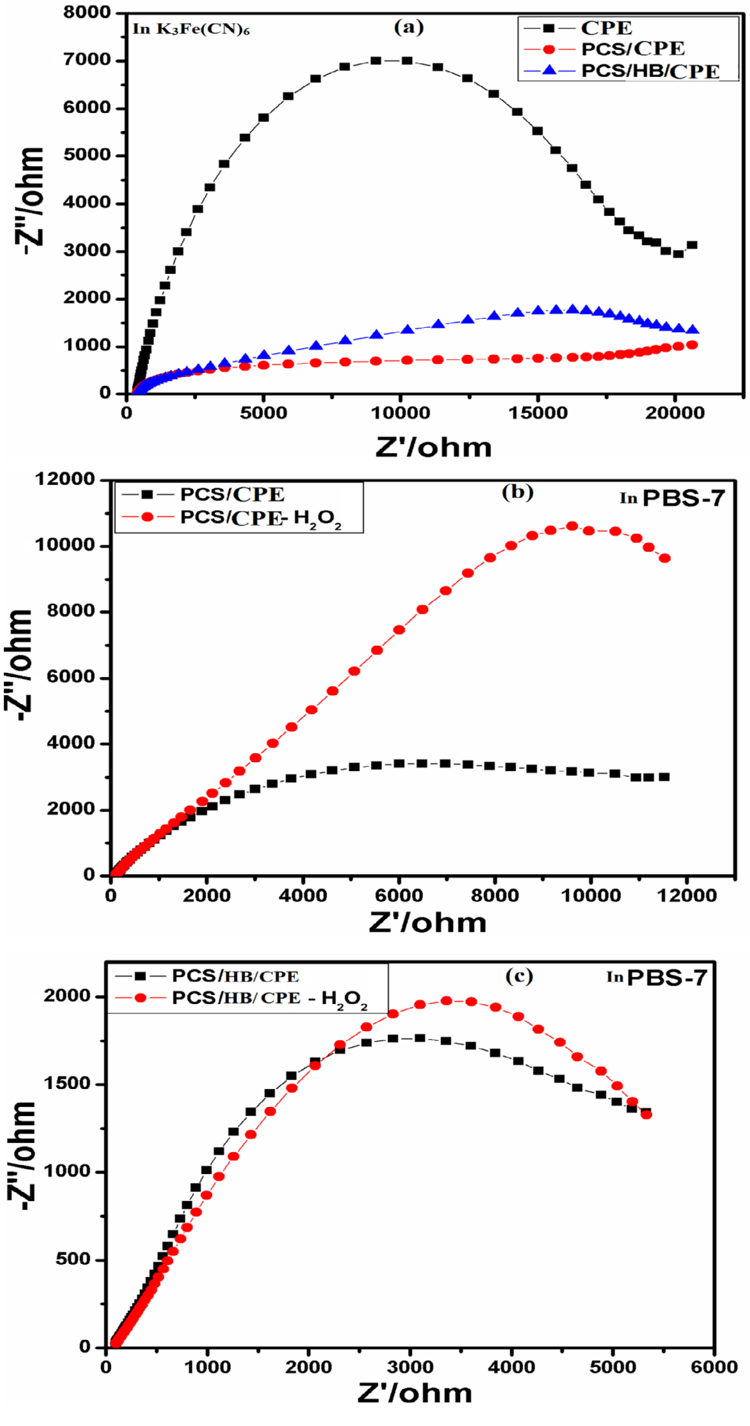

**Fig. 6.7** Electrochemical impedance spectra in 0.1 M PBS-7 (a) bare CPE, PCS/CPE, PCS/HB/CPE with $K_3Fe(CN)_6$(b) PCS/CPE with 0.32 mM $H_2O_2$, (c) PCS/HB/CPE with 0.32 mM $H_2O_2$

### 6.3.8 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-Ttyrosine, 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. 6.8). We observed the highest current response of hydrogen peroxide (225% increment with respect to blank). Interference study indicated no significant impact on the response of hydogen peroxide *(c.f. B5).*

### 6.3.9 Application for PCS/HB/Gox for Glucose Sensing:

We fabricated a carbon paste electrode by using PCS, HB and Glucose oxidase (GOx) to detect glucose. Enzymatic oxidation of glucose release hydrogen peroxide. We have comparatively studied the electrochemical sensing of glucose by using PCS/CPE, PCS/HB/CPE and PCS/HB/GOx/CPE in PBS-7 (fig. 6.9). Calibration curve was plotted by measuring cathodic current with respect to the concentration of glucose *(c.f. B6).* We observed that PCS/HB/GOD/CPE showed better response than others in terms of higher peak current and more reversible electrode kinetics.

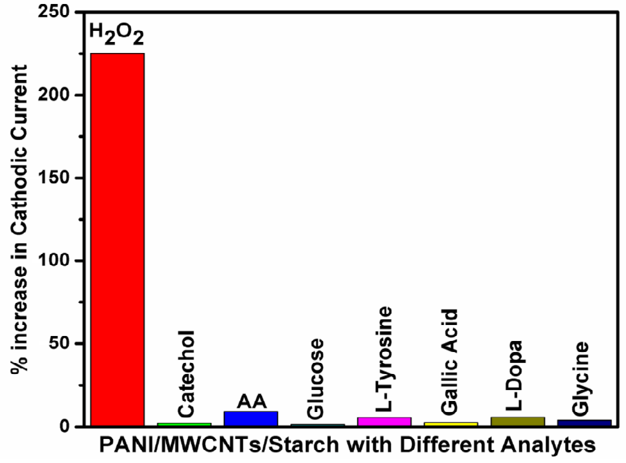

**Fig. 6.8** Percentage increase in cathodic current response with respect to blank for different analytes on [PANI/MWCNTs/Starch]-HB modified carbon paste electrode

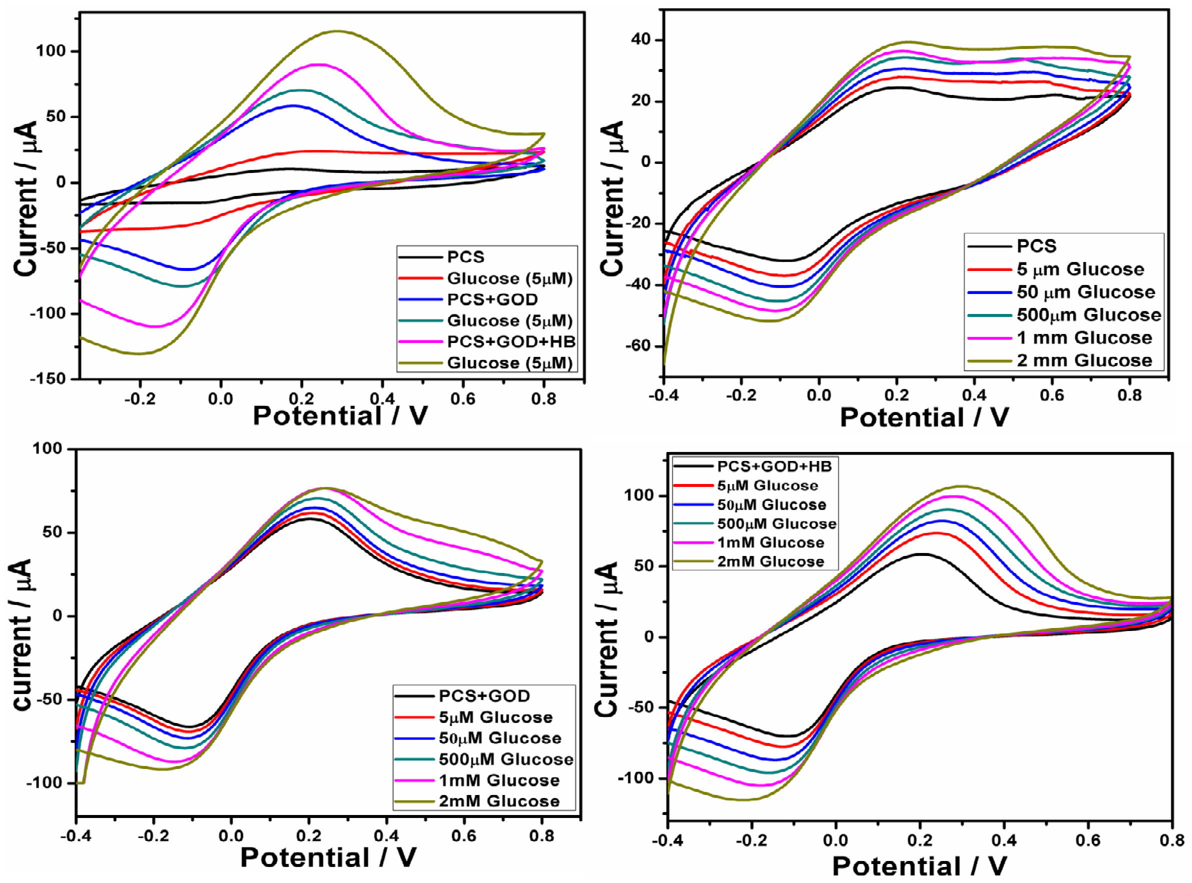

**Fig. 6.9** CV response of Glucose on PCS, PCS-HB, PCS/HB/God modified CPE

## 6.4 Conclusions:

We have successfully developed $H_2O_2$ electrochemical sensor material by using PCS-HB-CPE. The HB and PCS are compatible with each other and in combination they promote the reduction of hydrogen peroxide. ESI indicated that the proposed biosensor system has lower interfacial resistance than the bare electrode. The characteristics of the proposed biosensor are: 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 to $H_2O_2$. We have sucessfully detected glucose by using glucose oxidase along with PCS and HB modified carbon paste electrode. Work is under progress to develop water based electroactive ink formulation for low-cost sensor strips.