Design a hydrogen peroxide biosensor by use of catalase and modified carbon paste electrode with cadmium oxide nanoparticles

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ABSTRACT

In this research work, the electrochemical behavior of catalase/cadmium oxide nanoparticles/carbon paste electrode (CPE) was studied by cyclic voltammetry. A carbon paste electrode was modified chemically using cadmium oxide nanoparticles. Direct electron transfer of catalase (CAT) in the modified carbon paste electrode with cdo Nps was achieved easily. Electrochemical investigation indicated that a pair of well-defined quasi-reversible redox peaks of CAT heme Fe(III)/Fe(II) was obtained with the formal potential (E^O) located at -0.155 V (vs. SCE) in pH 7.0 phosphate buffer solution (PBS) 0.1 M. The fabricated CAT / CdO NPs/ CPE showed good electrocatalytic ability to the reduction of hydrogen peroxide (H_2O_2), which exhibited a potential application in fabricating a new kind of third generation biosensor. In case of the latter, an improved dispersion of the cadmium oxide nanoparticles upon electrode fabrication may greatly enhance their performance in biosensor activity.

Keywords: biosensor, catalase, cadmium oxide nanoparticles, H_2O_2

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INTRODUCTION

Nanotechnology is a relatively new and vast field. The increased presence of nanomaterials in commercial products such as cosmetics and sunscreens, dental fillings, photovoltaic cells, and water filtration and catalytic systems has resulted in a growing public debate on the toxicological and environmental effects of direct and indirect exposure to these materials[1-2]. At present, these effects are not completely elucidated. Nanotechnology involves the tailoring of materials at atomic level to attain unique properties, which can be suitably manipulated for the desired applications [3-5]. Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [6]. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging [7], sensing [8], targeted drug delivery [9] and gene delivery systems [10] and artificial implants [11]. Sensors are the devices, which are composed of an active sensing material with a

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signal transducer[12-14]. The role of these two important components in sensors is to transmit the signal without any amplification from a selective compound or from a change in a reaction. These devices produce any one of the signals as electrical, thermal or optical output signals that could be converted in to digital signals for further processing[15]. One of the ways of classifying sensors is done based on these output signals. Among these, electrochemical sensors have more advantage over the others because; in these, the electrodes can sense the materials, which are present within the host without doing any damage to the host system. On the other hand, sensors can be broadly classified in to two categories as chemical sensors and biosensors. The biosensors can be defined in terms of sensing aspects, where these sensors can sense biochemical compounds such as biological proteins, nucleotides and even tissues [16-19]. The history of biosensors started in 1962 with the development of enzyme electrodes by scientist Leland C. Clark[20]. Since then, research communities from various fields such as very large scale integration (VLSI), physics, chemistry, and material science have come together to develop more sophisticated, reliable, and mature biosensing devices[21]. Applications for these devices are in the fields of medicine, agriculture, biotechnology as well as the military and bioterrorism detection and prevention. Cadmium oxide is attracting tremendous attention due to its interesting properties like direct band gap of 2.3 eV. It is widely used in the applications like the preparation of cadmium-coated baths and manufacture of paint pigments. Cadmium sulphide is one of the most studied materials with a band gap of 2.43eV. It is primarily used in solar cell and a variety of electronic devices[22]. The photoconductive and electroluminescent properties of cadmium sulphide have been applied in manufacturing a variety of consumer goods. In the present paper, synthesis and characterization of cadmium oxide and cadmium sulphide nanoparticles has been studied that used as facile electron transfer[23]. Enzymes are biological catalysts in the form of proteins that catalyze chemical reactions in the cells of living organisms. As such, they have evolved – along with cells – under the conditions found on planet Earth to satisfy the metabolic requirements of an extensive range of cell types[24]. Catalases are some of the most efficient enzymes found in cells. Each catalase molecule can decompose millions of hydrogen peroxide molecules every second. The cow catalase shown here (PDB entry 8 cat) and our own catalases use an iron ion to assist in this speedy reaction[25-27]. The enzyme is composed of four identical subunits, each with its own active site buried deep inside. The iron ion, shown in green, is gripped at the center of a diskshaped heme group[28]. Catalases, since they must fight against reactive molecules, are also unusually stable enzymes. Notice how the four chains interweave, locking the entire complex into the proper shape. Catalase is an important enzyme of oxidoreductase family, which has been widely employed in biosensors for sensitive and selective H_2O_2 determination [29]. However, the selection of suitable electrode material and novel immobilization matrices with good electronic properties is essential to enhance the direct electron transfer between Catalase and the electrode surfaces. Previous studies emphasis the key roles played by the nanomaterials in promoting the direct electrochemistry of Catalase [30-32]. In this paper, we investigated electrochemical behavior of catalase enzyme by use of carbon paste electrode (CPE) and cadmium oxide nanoparticles. The reduction reactions of mentioned electrochemical behavior used as hydrogen peroxide (H_2O_2) biosensor.

## MATERIALS AND METHODS

2.1. Reagents
Catalase from bovine liver (40-45 units/ mg) was purchased from Sigma. The phosphate buffer solution (PBS) consisted of a potassium phosphate solution (KH_2PO_4 and K_2HPO_4 from Merck; 0.1 M total phosphate) at pH 7.0. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water.

2.2. Apparatus
Cyclic voltammetric experiments were performed with a model EA-201 Electro Analyzer (chemilink systems), equipped with a personal computer was used for electrochemical measurement and treating of data. A conventional three electrode cell was employed throughout the experiments, with bare or cadmium oxide nanoparticles modified carbon paste electrode (5.0 mm diameter) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode and a platinum electrode as a counter electrode. The phase characterization was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. Samples were measured and recorded using a TU-1901 double-beam UV–visible spectrophotometer were dispersed in toluene solution.

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2.3. Synthesis of CdO NPs

For production of cadmium oxide nanoparticles, the Cadmium acetate (6.66 g, 0.5 M) was dissolved in 100 ml water and ammonia solution was added to above solution drop wise until *p*H value of about 8 was reached with constant stirring. The white precipitate was formed and it was allowed to settle for 5-6 hour and then filtered and washed 3-4 times with water. It was dried at 100°C and then grinded. The resulting powder was calcined at 400°C for 2 hour. It turned into yellowish colour which confirmed the formation of CdO. Below equation shows the schematic diagram for the preparation of CdO nanoparticles.

$$(CH_3COO)_2 \; Cd \cdot H2O + 2NH_4OH \rightarrow Cd\,(OH)_2 + 2H_2O + 2CH_3COONH_4$$
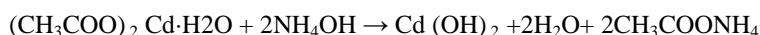
During calcinations as prepared powder loses H_2O, which is as follows:

$$Cd\,(OH)_2 \rightarrow CdO + H_2O$$
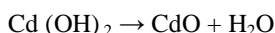
The temperature is 400 °C

2.4. Preparation of bare Carbon paste electrode

The carbon powder (particle size 50 mm, density 20-30g/100mL) was mixed with the binder, silicone oil, in an agate mortar and homogenized using the pestle. The electrode consisted of a Teflon well, mounted at the end of a Teflon tube. The prepared paste was filled into the Teflon well. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical contact with the external circuit. The electrode surface of the working electrode was renewed mechanically by smoothing some paste off and then polishing on a piece of transparent paper before conducting each of the experiments. The experiments were performed in unstirred solutions.

2.5. Preparation of Cdo nanoparticles modified carbon paste electrode

The Cdo nanoparticles modified carbon paste electrode was prepared by hand mixing of carbon powder, binder and 10 mg Cdo nanoparticle with silicon oil in an agate mortar to produce a homogenous carbon paste. Other steps of produced modified carbon paste electrode were similar to preparation of bare carbon paste electrode. A conventional three electrode cell was employed throughout the experiments, with bare or Cdo nanoparticles modified carbon paste electrode (4.0 mm diameter) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode and a platinum electrode as a counter electrode.
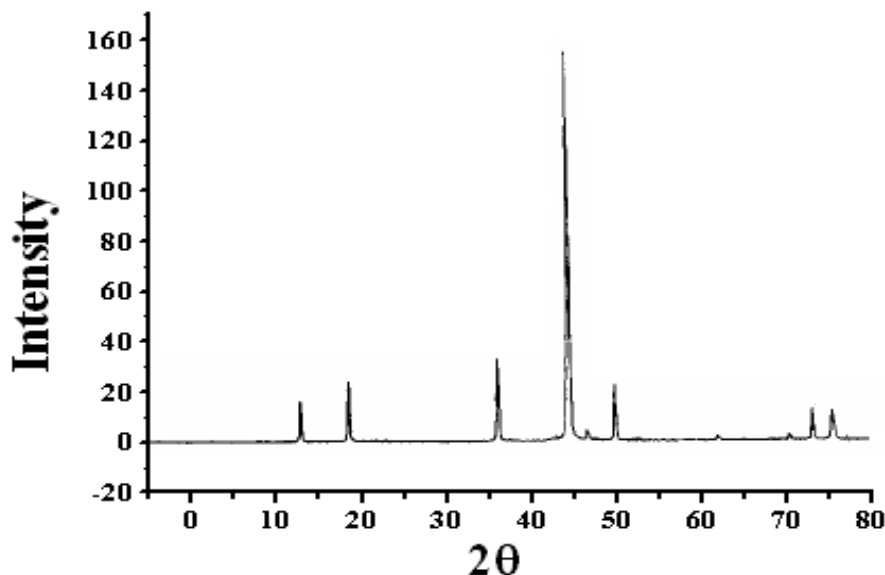

Figure 1. XRD pattern for CdO nanoparticles

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<div align="center">**RESULTS**</div>

3.1. X-ray diffraction of cadmium oxide nanoparticles

The XRD pattern for CdO nanoparticles was shown in Fig. 1 , the diffraction peaks are absorbed at 2θ values. The prominent peaks have been utilized to estimate the grain size of sample with the help of Scherrer equation [33] $D = K\lambda/(\beta \cos\theta)$ where K is constant(0.9), λ is the wavelength(λ = 1.5418 A°) (Cu Kα), β is the full width at the half-maximum of the line and θ is the diffraction angle. The grain size estimated using the relative intensity peak for CdO nanoparticles was found to be 30 nm and increase in sharpness of XRD peaks indicates that particles are in crystalline nature. The reflections are clearly seen and closely match the reference patterns for CdO (Joint Committee for Powder Diffraction Studies (JCPDS) File No. 05-0640).

3.2. UV–visible absorption spectra for CdO nanoparticles

The UV–visible absorption spectra of CdO nanoparticles was shown in Fig. 2; although the wavelength of our spectrometer is limited by the light source, the absorption band of the CdO nanoparticles have been shows a blue shift due to the quantum confinement in sample compare with bulk CdO particles. This optical phenomenon indicates that these nanoparticles show quantum size effect [34].
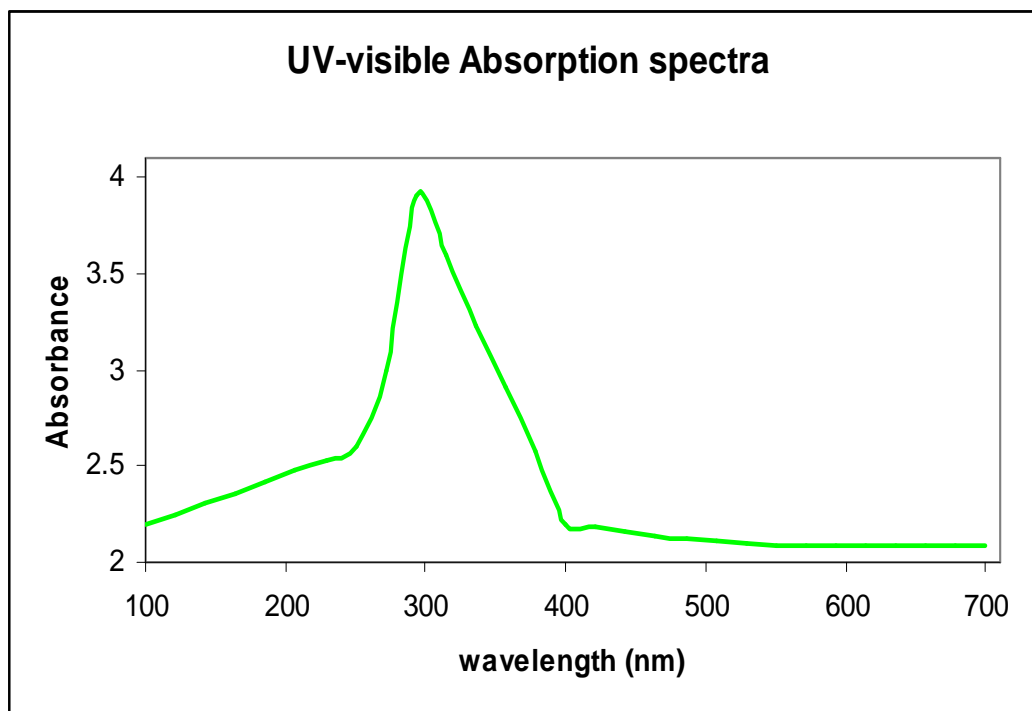

Figure 2. UV-Absorption spectra for CdO nanoparticles

### *3.3. Direct voltammetric behavior of the CAT/Cdo NPs/CPE electrode*

The integrity of the immobilized catalase construction and its ability to exchange electrons with the nanometer-scale Cdo particles surfaces were assessed by voltammetry. A macroscopic electrode was required to attain a large enough catalase sample to yield detectable direct oxidation and reduction currents. The comparative CVs for the Cdo NPs/CPE and CAT / Cdo NPs/ CPE electrodes in 0.1 M PBS (pH 7.0) were obtained. These voltammograms are demonstrated in Fig. 3 (a,b). From this Figure, it was noticed that there were no voltammetric response on Cdo NPs/carbon paste electrode (Fig. 3a), which, Fig. 3(b) depicts a well-defined pair of oxidation–reduction (redox) peaks, observed on the CAT / Cdo NPs carbon paste electrode at 100 mv/s scan rate value. The CAT / Cdo NPs/ carbon paste electrode presented the reductive peak potential at -0.19 V and the corresponding oxidative peak potential at +0.12 V (at 100 mV s^{-1}), illustrating the adsorbed catalase on the nanometer-scale cadmium oxide particle surfaces. The difference of anodic and cathodic peak potential values was ΔE = 0.07 V. These redox peaks

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were attributed to the redox reaction of the catalase electroactive center. The formal potential (E^0) for the catalase redox reaction on the CAT / Cdo NPs/carbon paste electrode was -0.155 V with respect to the reference electrode.
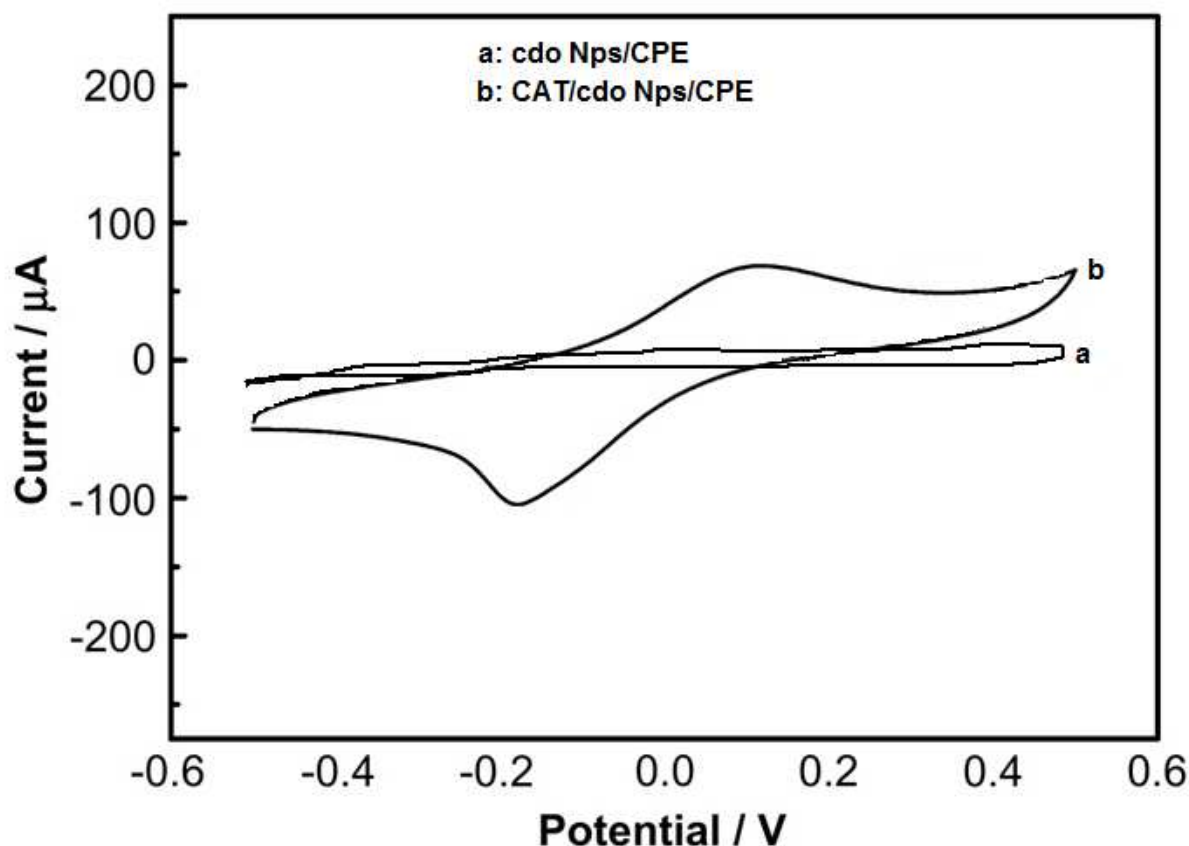
Figure 3.Cyclic voltammograms, using (a) the CdO NPs/CPE in 0.1 M phosphate buffer and (b CAT / CdO NPs/ CPE in 0.1 M phosphate buffer (scan rate: 100 mV/s).

The collected voltammograms in Fig. 4 (a), substantiated this statement that the nanometer-scale cadmium oxide particles could play a key role in the observation of the catalase CV response. On the grounds that the surface-to-volume ratio increases with the size decrease and because of the fact that the enzyme size is comparable with the nanometer-scale building blocks, these nanoparticles displayed a great effect on the electron exchange assistance between catalase and carbon paste electrode. To further investigate the catalase characteristics at the CAT/CdO NPs/CPE electrode, the effect of scan rates on the catalase voltammetric behavior was studied in detail. The baseline subtraction procedure for the cyclic voltammograms was obtained in accordance with the method reported by Bard and Faulkner [35]. The scan rate (v) and the square root scan rate ($v^{1/2}$) dependence of the heights and potentials of the peaks are plotted in Fig. 4(b) and Fig. 4 (c) respectively. It can be seen that the redox peak currents increased linearly with the scan rate, the correlation coefficient was 0.9973 (ipc = -0.293v - 93.1) and 0.9999 (ipa = 0.360v +34.25), respectively. This phenomenon suggested that the redox process was an adsorption-controlled and the immobilized catalase was stable. In Fig. 4 (c) It can be seen that the redox peak currents increased more linearly with the v in comparison to that of $v^{1/2}$.

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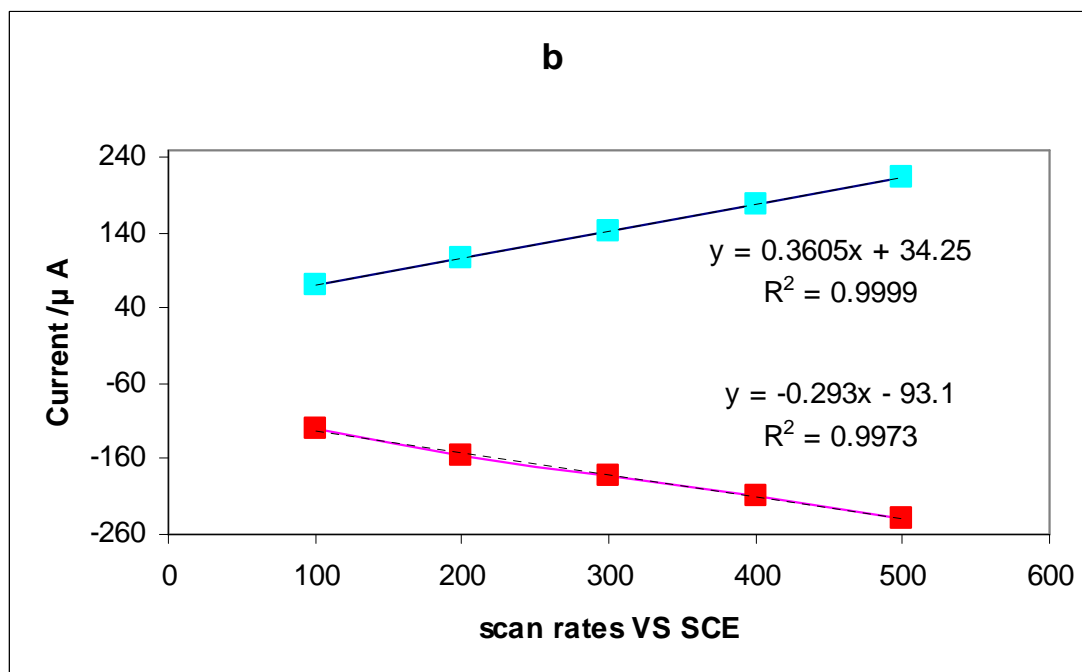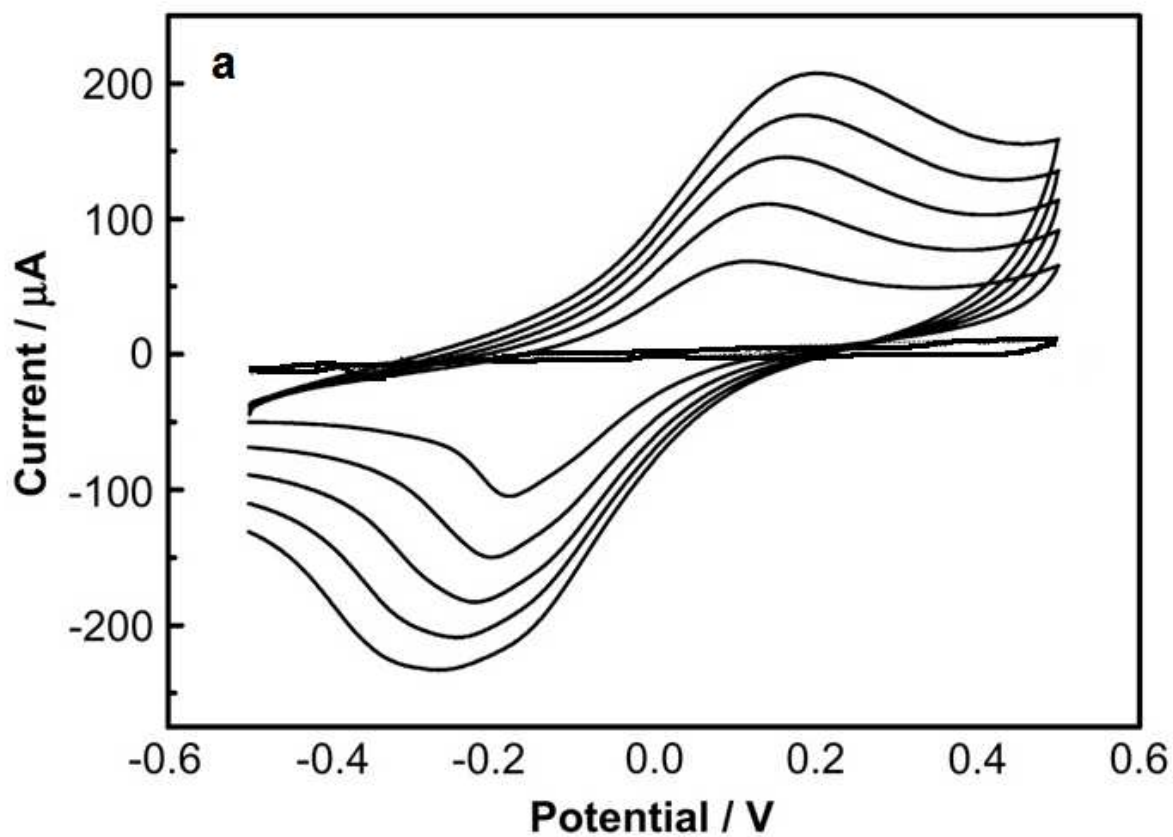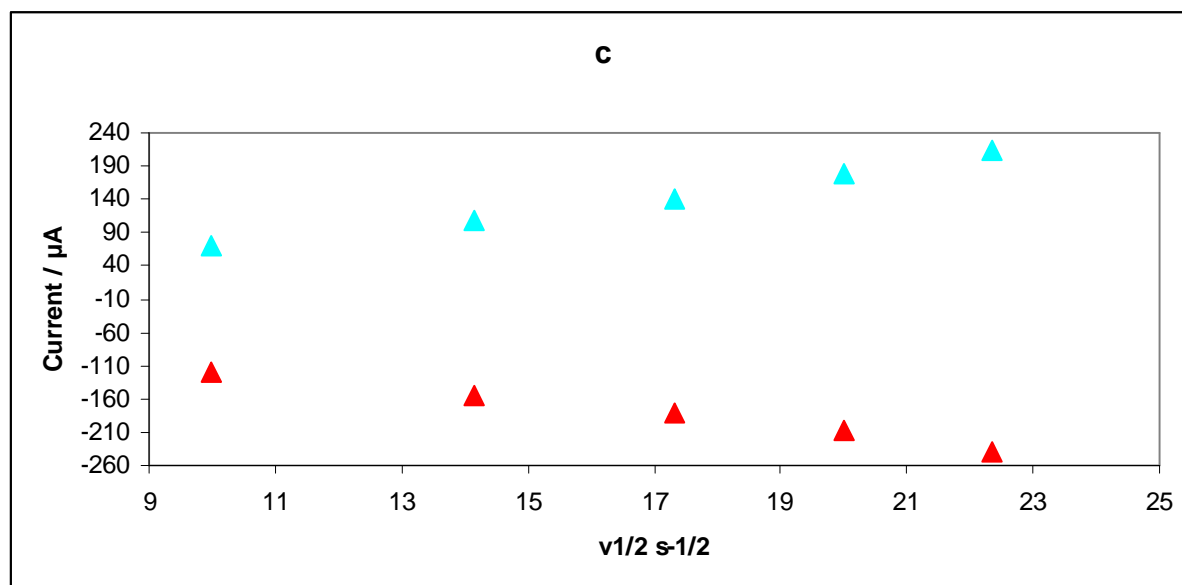
Figure.4. (a) CVs of CAT / CdO NPs/ CPE electrode in PBS at various scan rates, from inner to outer; 100, 200, 300,400 and 500 mV s^{-1}, the relationship between the peak currents (ipa, ipc) vs., (b) the sweep rates and (c) the square root of sweep rates.
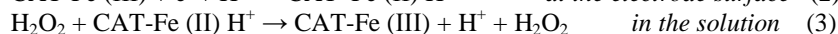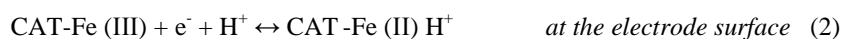
However, there is clearly a systematic deviation from linearity in this data, i.e. low scan rates are always on one side of the line and the high scan rate points are on the other. The anodic and cathodic peak potentials are linearly dependent on the logarithm of the scan rates (v) when $v > 1.0$ V s^{-1}, which was in agreement with the Laviron theory, with slopes of -2.3RT/αnF and 2.3RT/ (1 -α) nF for the cathodic and the anodic peak, respectively [36]. So, the charge-transfer coefficient (α) was estimated as 0.49. Furthermore, the heterogeneous electron transfer rate constant (ks) was estimated according to the following equation [36-37]:

$$[\log ks = \alpha \log(1-\alpha) + (1-\alpha) \log\alpha - \log\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta EP}{2.3\,RT}] \tag{1}$$

Here, n is the number of transferred electrons at the rate of determining reaction and R, T and F symbols having their conventional meanings. The average heterogeneous transfer rate constant (ks) value was calculated about, 1.23 s^{-1}.

3.4. Electrocatalytic reduction of H_2O_2 on the CAT/Cdo NPs/CPE retained electrode
Upon addition of H_2O_2 to 0.1M pH 7.0 PBS, the cyclic voltammogram of the CAT/Cdo NPs/CPE electrode for the direct electron transfer of catalase changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 5a), while the change of cyclic voltammogram of bare or Cdo Nps/ CPE was negligible (not shown), displaying an obvious electrocatalytic behavior of the catalase to the reduction of H_2O_2. The decreases of the oxidative peak current together with the increases of the reductive CAT/Cdo NPs/CPE. The electro-catalytic process could be expressed as follows:

CAT-Fe (III) + e$^-$ + H$^+$ ↔ CAT -Fe (II) H$^+$ *at the electrode surface*   (2)
H_2O_2 + CAT-Fe (II) H$^+$ → CAT-Fe (III) + H$^+$ + H_2O_2 *in the solution*   (3)

calibration curve (Figure 5b) shows the linear dependence of the cathodic peak current on the H_2O_2 concentration in the range of 25 to 210 µM. In Figure 5 b, at higher concentration of H_2O_2, the cathodic peak current decreased and remains constant. Upon addition of an aliquot of H_2O_2 to the buffer solution, the reduction current increased steeply to reach a stable value (Fig 5 b). This implies electrocatalytic property of electrode. Thus, this experiment has introduced a new biosensor for the sensitive determination of H_2O_2 in solution.
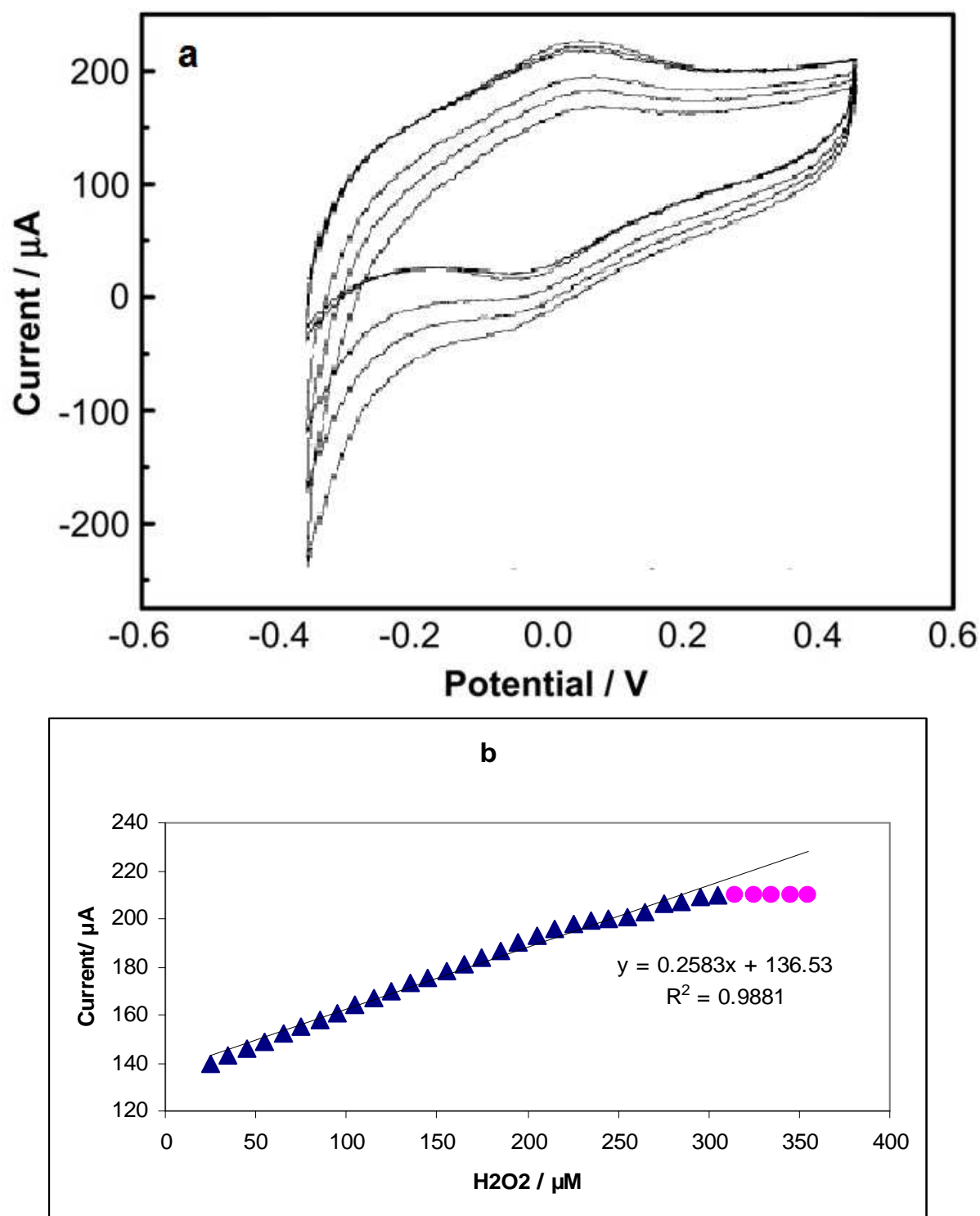
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Figure .5. (a) Cyclic voltammograms obtained at an CAT / CdO NPs/ CPE in 0.1M phosphate buffer solution (pH 7.0) for different concentrations of and (b) the relationship between cathodic peak current of CAT and different concentrations of H$_2$O$_2$ (scan rate: 100 mVs^{-1}).

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3.5. Effect of temperature on the H_2O_2 sensor

Temperature is an important parameter affecting the electrocatalytic activity of enzyme or protein. Fig. 6 shows the effect of temperature on sensor response. With an increasing temperature from 5 ^{o}C to 50 ^{o}C the response and the electrocatalytic activity of the immobilized catalase increased. The immobilized catalase had activity even at 50 ^{o}C. It was evident that the immobilized catalase had good thermal stability because of the unchanged ability of microenvironment and its native structure upon temperature change. These results indicated that this sensor could handle in a wide range of temperature, but it has maximum of response only at 25°C.
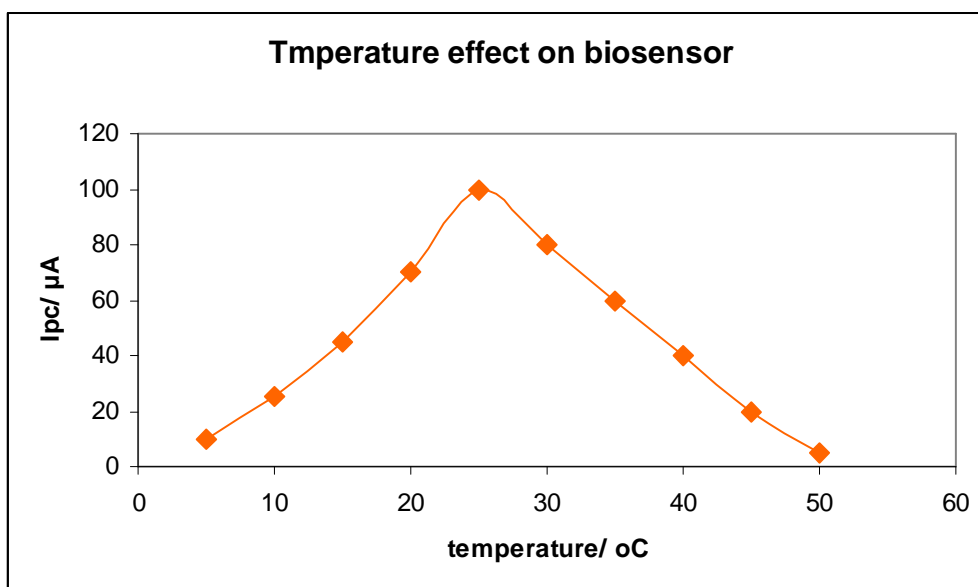

Figure. 6. Effect of temperature on biosensor at pH 7.0.

3.6. Stability of the H_2O_2 biosensor

The stability of CAT / CdO NPs/ CPE electrode biosensor has been checked by carrying out experiments at the regular interval of a week and it has been found that CAT / CdO NPs/ CPE electrode based electrochemical biosensor retains its 93% activity after 18 days. The loss in the activity of biosensor is not due to the denaturation of catalase but it is due to the poor adhesion of cadmium oxide Nanoparticles on the carbon paste electrode. For a result, interface materials have not high effect on operation of this biosensor. Undoubtedly, nanotechnology in combination with bioelectrochemistry can extremely influence the development rate of these scientific fields[10-12]. However, a number of challenges remain to be faced, which are related to the processing of the electrode modifications in a more controlled method. The charge transport mechanism in the nanostructured biointerfaces presents a great interest, requiring further investigation. Nevertheless, the recent advances have been important for the comprehension of the nanostructured biointerfaces. As a result, it will be possible to study the modern material sciences, including bioelectronics, biocatalysis and biosensing.

CONCLUSION

Carbon paste electrodes modified with nanoparticles of cadmium oxide were employed for the electrocatalytic reduction and determination of hydrogen peroxide. Catalase can be effectively immobilized on cadmium oxide nanoparticles modified carbon paste electrode to produce a fast direct electron transfer. The immobilized catalase maintains its bioactivity and native structure. The combination of catalase enzyme and Cdo NPs in the biosensor would result in the improvement of analytical performance, characterized by broader linear range and lower detection limit for H_2O_2 determination. Moreover, the biosensor possesses good stability and reproducibility, and achieves 93% of the steady state.

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