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ORIGINAL ARTICLE

DESIGN A HYDROGEN PEROXIDE BIOSENSOR BY USE OF CATALYSES AND MODIFIED GLASSY CARBON ELECTRODE WITH CADMIUM OXIDE NANO PARTICLES AND ENZYME CATALYSES

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ABSTRACT: In this research we have identified new biosensor to measure hydrogen peroxide using catalase enzyme and glassy carbon electrode. Electrode modified by scanning electron microscope and transmission electron microscope was surveyed. Direct electrochemistry of Catalase in glassy carbon electrode was obtained easily and a couple of similar returnable peaks was presented from iron II and III with formal potential (E) about 48mV/ Ph that shows linear relationship between formal potential of the mentioned electrode and Ph between 5-11. This electrochemical process was performed in *Phosphate buffered saline* in Ph=7 and scanning speed of 100Mv/s . Designed sensor has high sensitivity and short responding time and can be used to determine concentration of hydrogen peroxide in linear range of 50-350 mmol. Also this sensor has very good stability. We synthesized the cadmium Nanoparticles chemically in laboratory and in the next stage studies of these Nanoparticles using x-ray diffraction (XRD) approved the claim that synthesized nanoparticles are cadmium oxide. Uv-vis spectrum of cadmium oxide nanoparticles showed absorption in 380nm area.

KEYWORDS: Catalase Enzyme, Cadmium Oxide Nanoparticles, Glassy Carbon Electrode, Hydrogen Peroxide.

INTRODUCTION

Nanotechnology is a relatively and vast field.The increased present of nano materials in commercial products dental fillings, photovoltaic cell and catalytic systems. Nanotechnology involves the tailoring of materials at atomic to attain unique properties. Most of the natural processes also take place in the nanometer seal regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems. Sensor are the devices, the role of these two important compounds 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 into digital signal for further processing. The history of biosensors started in 1962 with the development of enzyme electrodes by scientist Leland C.clark. Since then research communities from various fields such as very large scale integration physics, chemistry, and material science have come together to develop more devices. Cadmium oxide and cadmium solphide nanoparticles have been studied that used as facile electron transfer. Enzyme is biological catalysts in the form of proteins that catalyze chemical reaction in the cell of living organism. Catalyses are some of the most efficient enzymes found in cell, each catalyses molecule can decompose millions of hydrogen peroxide molecules every second. The enzyme is composed of four identical subunits. The iron ion, shown in green, is gripped at the center of a disk shaped home group. Catalyses, since they must fight against reactive molecules, are also unusually stable enzyme. Notice how the four chains interwave, looking the entire complex into the proper shap. Catalyses are an important enzyme of oxide reductase family which has been widely employed in biosensor for sensitive and selective H2O2 determination. However the selection of suitable electrode material and novel immobilization matrices with good electronic properties is essential to enhance the direct electron transfer between catalyses and electrode surface.Provious studies emphasis the key roles played by the nanomaterial in promoting the direct electrochemistry of catalyses. Peroxides exhibit bleaching, oxidizing and catalytic abilities, bence their widespread use as industrial reagents. H2O2 is the main bleaching agent in detergents, pulp and paper, cellulose, domestic bleach. Textiles and wine cork manufacturing and also in the food industry. It is used in small quantities as a mild disinfectant and antiseptic in pharmaceutical and cosmetic products. Some of these products such as tooth paste, teeth whitener and mouth

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wash are for application According to the reaction given below, hydrogen peroxide is converted into oxygen by catalyses dispersed in the gelatin layer and the amount oxygen produced can then be detected amperometrically:

$$H2O2 \rightarrow \frac{(catalase)}{} \frac{1}{2} O2 + H2O$$

We investigated electrochemical behavior of catalyses enzyme by use of glassy carbon electrode and cadmium oxide nano particles. The reduction reactions of mentioned electrochemical behavior used as hydrogen peroxide biosensor.

MATERIALS AND METHODS

2.1. Reagents

Catalyses from bovine liver were purchased from sigma. The phosphate buffer solution (PBS) consists of potassium phosphate solution (KH2PO4) and sodium phosphate solution(NazHpo4) from merk,(0.1m total phosphate)at PH:7.All other chemicals were of analytical grade and without further purification cadmium sulphat, sodium hydroxide, acetic acid, ethanol, toloen and sodium chloride. All solutions were made up with doubly distilled water

2.2. Apparatus

Cyclic volt metric experiment were performed with a model Potentiostat/Galvanostat.Electrode analyses, a conventional three electrode cell was employed throughout the experiments with bare or cadmium oxide nanoparticles modified glassy carbon electrode as a working electrode, a saturated calomel electrode as a reference electrode and a platinum electrode as a center electrode. The phases characterization was performed by means of x-ray diffraction (XR) using a D/max-RA diffractomer cukα redition. Samples were measured and recorded using a TU-90 double-beam UV-visible spectrophotometer was dispersed in toulen solution.

2.3. Synthesis of cdo NPS

Ethanol was dried using type 3A.molecular.Sieves before use NaoH pallets were pulverized into fine powder under a dry use nitrogen flow. In a typical experiment first solution prepared using 0/03m cdSO4, 0/06m CH3COOH and 40mgctAB as surfactant in ldm ^{3} of duble distilled water.

The second solution was prepared by 0/09m NaoH pallets and 25ml 70% ethanol in ldm^3 of double distilled water. Then first solution was added to second solution. The obtained precipitate was filtered by using whatmann filter paper and dried at 80˚c in hot air oven about 1 hour then dried precipitate was transferred to silica crucible and ignited at 400˚c for about 4 hours. Then obtained powder was washed with ethanol three to four times to remove impurities present in the particles. Then these are characterized using XRD, UV-visible absorption spectroscopy and applied for fabrication of glassy carbon electrode.

RESULTS

3.1. X-ray diffraction of cadmium oxide NPS

The XRD pattern for cdo nanoparticles was show in Fig α, the diffraction peaks are absorbed at 20 values. The prominent peak have been utilized to estimate the grain size of sample with help of Scherer equation.(D=kʎ/BcosΘ)where K is constant(0/9),ʎ is the wavelength (ʎ=1/54)A˚,cukα,B is the full width at half maximum of the line and Θ is the diffraction angle. The grain size estimated using the relative in tensity peak for cdo nanoparticles were found is 42/8nm 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 committee for powder Diffraction studies.

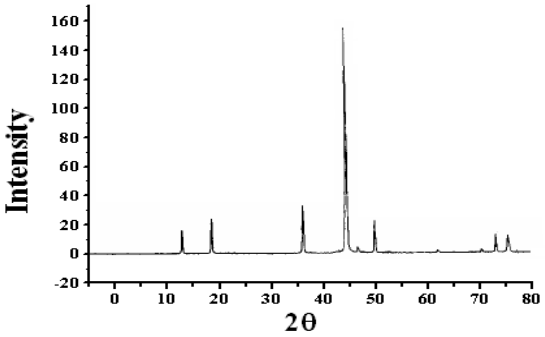

Figure 1: X-ray diffraction of cadmium oxide NPS

3.2. UV-Visible absorption spectra for cdo nanoparticles "cdo nanoparticles"

The UV-visible absorption spectra of cdo nanoparticles were shown in Figz. Although the wavelength of our spectrometer is limited by the light source, the absorption band of the cdo nanoparticles have been shown a blue shift due to the quantum confinement in sample compare with bluk cdo particles. This optical phenomenon indicates that this nanoparticles show quantum size effect.

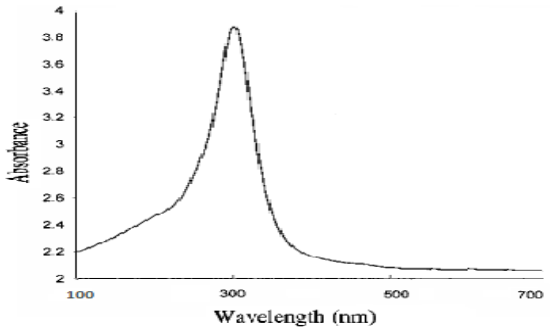

Figure 2: UV-Visible absorption spectra for cdo nanoparticles "cdo nanoparticles"

3.3. The average diameter of the synthezd cdo nanoparticle

The average diameter of the synthezd cdo nanoparticle is about 42/8nm, and has a very narrow particle distribution. This statement illustrated in Figure3.Show a SEM picture of the cdo nanoparticles.

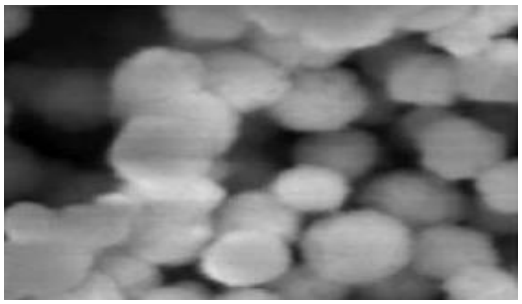

Figure 3: The average diameter of the synthezd cdo nanoparticle

3.4. Direct volt metric behavior of the CAT/cdoNPS/G.C.E

The integrity of the immobilized catalyses construction its ability to exchange electron with the nanometer scale cdo particles surface were assessed by voltametry A macroscopic electrode was required to attain a large enough catalyses sample to yield detectable direct oxidation and reduction current. The comparative cvs for the cdo/CAT/G.C.E in 0/1m PBS (ph: 7) were obtained these voltamograms are demonstrated in Fig4 (a&b) from this figure. It was noticed that there were no volt metric response on Cdo/G.C.E (a).fig4 (b) depicts a well defined pair of oxidation-reduction (redox) peaks, observed on the CAT/cdoNPS/G.G.E at 100mv/S scan rate value. The CAT/cdoNPS/G.C.E presented the reductive peak potential at 140mv and corresponding oxidative peak potential at 175mv illustrating the absorbed catalyses on the nanometer. Substantiated this statement that the nanometer scale cadmium oxide particles could play a key role in the observation of the catalyses CV response on the grounds that the surface to volume ratio increase with the size decrease and because of the fact that the enzyme size comparable with the nanometer building block. These nanoparticles displayed a great effect on the electron exchange assistance between catalyses and glassy carbon electrode. The effect scan rate on the catalyses volt metric behavior was studied in detail. The baseline and subtraction procedure for the cyclic voltamograms was obtained in accordance of the heights and potential of the peak are plotted It can be seen that the redox peak current increased lineary with the scan rate. The correlation coefficient was ipc=994% and ipa=992% respectively. This phenomenon suggested that the redox process was on absorption-controlled and the immobilized catalyses were stable.

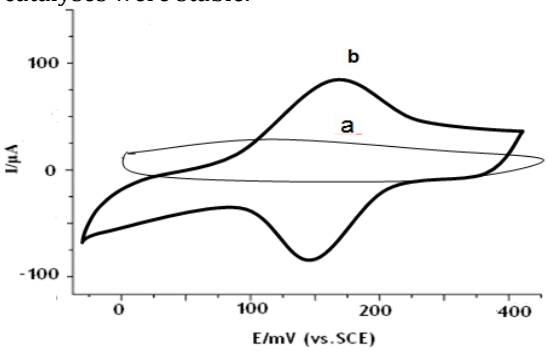

Figure 4: Direct volt metric behavior of the CAT/cdoNPS/G.C.E

3.5. Electro catalytic reduction of H202 on the CAT/cdoNPS/G.C. electrodes

Upon addition of H2O2 to 0/1m PH:7(PBS),the cyclic voltamogram of the CAT/cdoNPS/G.C.E for the direct electron transfer of catalyses change dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig6)while the change of cyclic voltamogram of bare or NPS cdo/G.C.E was negligible displaying and obvious electro catalytic behavior of the catalyses to the reduction of H2O2.The decrease of the oxidative peak current together with the increase of the catalytic process could be expressed as followers.

CAT- Fe(III) + e^- + H^+ ↔ CAT-Fe(II)H^+

H_2O_2 + CAT-Fe (II) H^+ → CAT-Fe (III) H^+ + H_2O_2

Dependence of the cathodic peak current on the H2o2 concentration of in the range of 50-350mm higher concentration of H2O2 to the buffer solution, the reduction current increase steeply to reach a stable value (5).This implies electro catalytic property of electrode.

Thus, this experiment has introduced a new biosensor for the sensitive determination of H2O2 in solution.

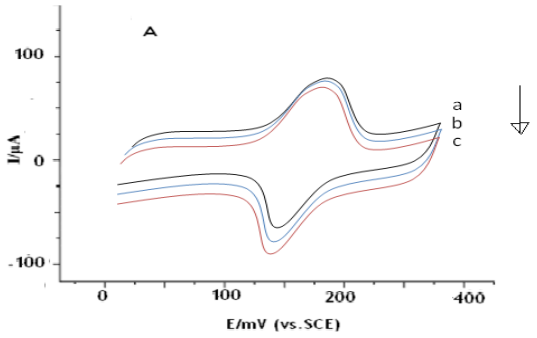

Figure 5: Electro catalytic reduction of H2O2 on the CAT/cdoNPS/G.C. electrodes

3.6. Effect of temperature on the H2O2 sensor

Temperature is an important parameter affecting the electro catalytic activity of enzyme or protein (Fig6) show the effect of temperature or sensor response with an increase temperature from 5˚c to 50˚c the response and the electro catalytic activity of the immobilized catalyses increase. The immobilized catalyses had activity even at 50˚c.It was evident that the immobilized catalyses had good thermal stability because of the unchanged ability of microenvironment and its native. Structure upon temperature change These result indicated that this sensor could handle in a wide range of temperature , about it has maximum of response 20-30˚c.

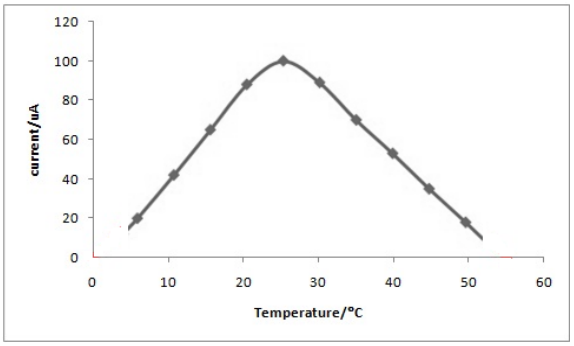

Figure 6: Effect of temperature on the H2O2 sensor

3.7. Stability of the H2O2 biosensor

The stability of CAT/cdoNPS/G.C.E biosensor has been checked by carrying out experiments at the regular interval of a week and it has been found that CAT/cdoNPS/G.C.E based electrochemical biosensor retain its 87% activity after 30 days. The loss in the activity of biosensor is not due to the denaturation of catalyses but it is due to the poor adhesion of cadmium oxide nanoparticles on the glassy carbon electrode.

CONCLUSION

Glassy carbon electrode modified with nanoparticles of cadmium oxide was employed for the electro catalytic reduction and determination of hydrogen peroxide. Catalyses can be effectively immobilized on cadmium oxide nanoparticles modified glassy carbon electrodes to produce a fast direct electron transfer. The immobilized catalyzes maintain its bioactivity and native structure. The combination of catalyzes enzyme and cdoNPS in the biosensor would result in the improvement of analytical performance, characterized by broader linear range and lower detection limit for H2O2 determination.

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