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ORIGINAL ARTICLE

DESIGNING A BIOSENSOR FOR DETERMINATION OF PARAOXON BY USING GOLD ELECTRODE MODIFIED WITH CUO NANO PARTICLES AND ACETYLCHOLINESTERASE

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ABSTRACT: Pesticides are extensively used in the modern agriculture as insecticide due to their high efficiency. Unfortunately these compounds have acute toxicity and can harm to human health and the environment. So in furtherance of environment protection purposes and to secure human health safety it is necessary to develop the sensitive and rapid technologies for detection of pesticides residue. Biosensor can be replaced by today analyses methods like liquid chromatography, gas chromatography and enzyme - linked immune absorption measure by simplifying or removing sample preparation in result of decrease in analysis time and cost. Among these sensors enzyme based electrochemical biosensors are absorbing especially due to some specifications like high sensitivity, rapid reaction and miniature size that presented them as a promising alternative for rapid detection of pesticides. In this research we have provided possibility to measure and detect concentration of paraoxon poison through chemical electrode by Modifying gold electrode level with copper oxide nanoparticles and acetylcholinesterase and also we have studied the electrochemical behavior of this protein structure. We studied copper oxide nanoparticles synthesized in the laboratory chemically by using the spectrums Uv-vis, TEM, XRD and SEM and was confirmed by using the spectrums. Our synthesized nanoparticles are copper oxide and spectrophotometer device showed absorption in the 270 nm area for nanoparticles. It was determined by using TEM and SEM that our nanoparticles are in the form of circle and their size is 20-40 nm. Direct electrochemistry of the acetylcholinesterase was obtained in the gold electrode and analyzable and clear peaks were presented. Enzyme inhibition by paraoxon shows linear performance of its concentration in two ranges of 0.1-1.80 and 3-10 mmol. This electrochemical process is performed in phosphate buffered saline and in PH=7. This biosensor can be used to determine paraoxon due to its suitable characteristics and easy preparation.

KEYWORDS: Acetylcholinesterase Enzyme, Copper Oxide Nanoparticles, Gold Electrode, Paraoxon Poison

INTRODUCTION

Nanotechnology involves the study, manipulation, creation and use of materials, devices and systems typically with dimensions smaller than 100 nm. Nanotechnology is playing an increasingly important role in the development of biosensors Sensitivity and other attributes of biosensors can be improved by using nanomaterials in their construction. Nanomaterials, or matrices with at least one of their dimensions ranging in scale from 1 to 100 nm, display unique physical and chemical features because of effects such as the quantum size effect, mini size effect, surface effect and macro-quantum tunnel effect. Use of nanomaterials in biosensors allows the use of many new signal transduction technologies in their manufacture. Because of their submicron size, nanosensors, nanop- robes and other nanosystems are revolutionizing the fields of chemical and biological analysis, to enable rapid analysis of multiple substances in vivo. Here we review the major aspects of the nanotechnology-based biosensors. Pesticides are widely used in modern agriculture due to their high efficiency as insecticides .Unfortunately, these compounds exhibit high acute toxicity, with the majority being hazardous to both human health and the environment . Therefore, for human health safety and environmental protection purposes, it is necessary to develop sensitive and fast detection technology for pesticide residues. Biosensors can substitute the current analytical methods (liquid chromatography , gaschromatography, enzyme-linked immunoabsorbant assays by simplifying .Among them, the enzyme-based electrochemical biosensors are particularly attractive because of their high sensitivity, rapid response and miniature size, which have emerged as a promising alternative to rapidly detect pesticides.Nanoparticles have numerous possible application in biosensors. For

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example, func- tional nanoparticles (electronic, optical and magnetic) bound to biological molecules (e.g. peptides, proteins, nucleic acids) have been developed for use in biosensors to detect and amplify various signals. Some of the nanoparticle-based sensors include the acoustic wave biosensors, optical biosensors, magnetic and electrochemical biosensors, as discussed next.

Acetylcholine-mediated neurotransmissionis fundamental for nervous system function. Its abrupt blockade is lethal and its gradual loss, as in Alzheimer's disease, multiple sys- tem atrophy and other conditions , is asso- ciated with progressive deterioration of cog- nitive, autonomic and neuromuscular functions. Acetylcholinesterase (AChE) hydrolyses and inactivates acetyl- choline, thereby regulating the concentration of the transmitter at the synapse. Termination of activation is normally depen- dent on dissociation of acetylcholine from the receptor and its subsequent diffusion and hydrolysis, except in diseases where acetyl-choline levels are limiting or under AChE inhibition, conditions that increase the duration of receptor activation.We investigated electrochemical behavior of Acetylcholinesterase enzyme by use of gold electrode and Copper oxide nano particles. The reduction reactions of mentioned electrochemical behavior used as Paraoxon Poison biosensor.

MATERIALS AND METHODS

2.1. Reagents

Acetylcholinesterase enzyme were purchased from sigma. The phosphate buffer solution (PBS) consists of potassium phosphate solution (KH_2PO_4) and sodium phosphate solution (Na_2Hpo_4) from merk,(0.1m total phosphate)at PH:7.All other chemicals were of analytical grade and without further $(CH_3COO)_2$ Cu $.H_2O$ NaOH,NaCl, Na_2HPO_4. $2H_2O$, H_2O paraoxon, acetyltiocholin .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 copper oxide nanoparticles modified gold 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.

RESULTS

3.1. Synthesis of cuo NPS

3.1.1. X-ray diffraction of copper oxide NPS

The XRD pattern for cuo nanoparticles was show in Fig 1, 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 35nm 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 cuo committee for powder Diffraction studies (Figure 1)

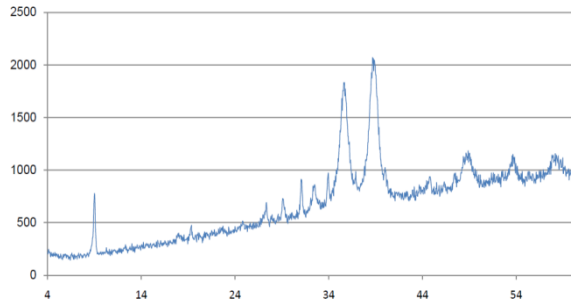

Figure 1: The reflections are clearly seen and closely match the reference patterns for cuo committee for powder Diffraction studies

3.1.2. UV-Visible absorption spectra for cuo nanoparticles "cuo nanoparticles"

The UV-visible absorption spectra of cuo nanoparticles were shown in Fig 2. Although the wavelength of our spectrometer is limited by the light source, the absorption band of the cuo nanoparticles have been shown a blue shift due (270nm) to the quantum confinement in sample compare with bluk cuo particles. This optical phenomenon indicates that this nanoparticles show quantum size effect.

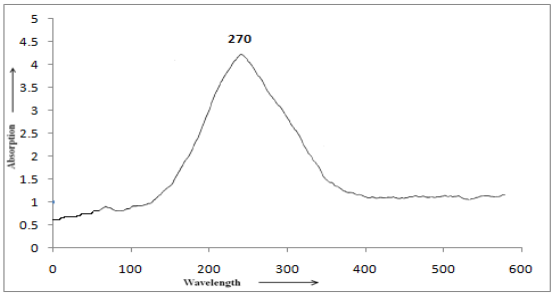

Figure 2: The UV-visible absorption spectra of cuo nanoparticles

3.1.3. The average diameter of the synthezd cuo nanoparticle

The average diameter of the synthezd cuo nanoparticle is about 40nm, and has a very narrow particle distribution. This statement illustrated in Figure3, Figure 4. Show a SEM picture and TEM picture of the cuo nanoparticles.

Figure 3: SEM picture and TEM picture of the cuo nanoparticles

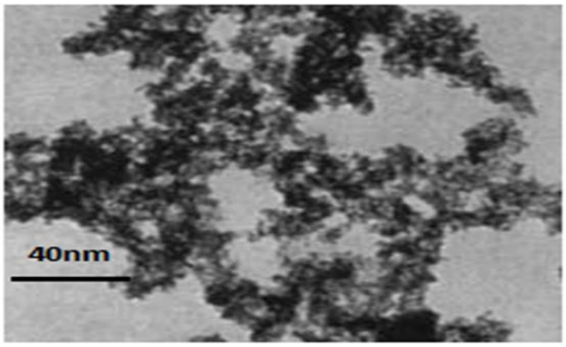

Figure 4: The average diameter of the synthezd cuo nanoparticle

3.1.4. Direct volt metric behavior of the TCh/CuO NPS/G.E

The integrity of the immobilized Acetylcholinesterase construction its ability to exchange electron with the nanometer scale CuO particles surface were assessed by voltametry A macroscopic electrode was required to attain a large enough Acetylcholinesterase sample to yield detectable direct oxidation and reduction current. The comparative cvs for the CuO/AChE/G.E in 0/1m PBS (ph: 7) were obtained these voltamograms are demonstrated in Fig 5 (a&b) from this figure. It was noticed that there were no volt metric response on CuO/G.E (a). fig 5 (b) depicts a well defined pair of oxidation-reduction (redox) peaks, observed on the AChE/cuo NPS/G.E at 100mv/S scan rate value. These nanoparticles displayed a great effect on the electron exchange assistance between Acetylcholinesterase and gold electrode. The effect scan rate on the Acetylcholinesterase 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 0/81 v current increased lineary with the scan rate.

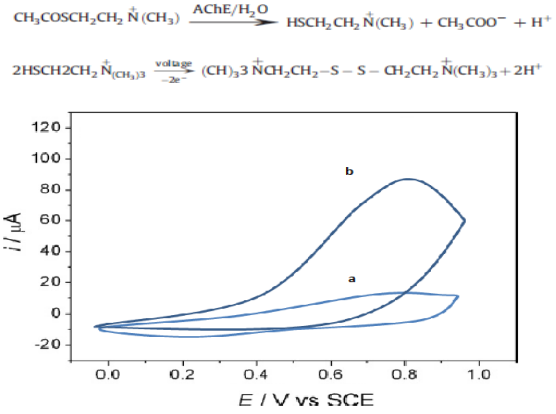

Figure 5: Direct volt metric behavior of the TCh/CuO NPS/G.E

3.1.5. Electrochemical detection of pesticides

The AChE/CuO /G.E biosensor employed for the determination of pesticides using differential pulse voltammetry (DPV) method. The performance of the biosensor was tested by its DPV response in pH 7 PBS solution containing 0/1 mM ATCh. Then the electrode was rinsed with water and incubated in an aqueous solution containing the desired concentration of paraoxon for 10 min. The inhibition rate of pesticides was calculated as follows:

Inhibition % ¼ IP,control IP,exp =IP,control 100%

where IP,control was the peak current of ATCh on AChE/cuo/G.E with pesticides inhibition, IP,exp was the peak current of ATCh on AChE/cuo/G.E pesticides inhibition. Inhibition (%) was plotted against the concentrations of the pesticides to obtain linear calibration graphs.

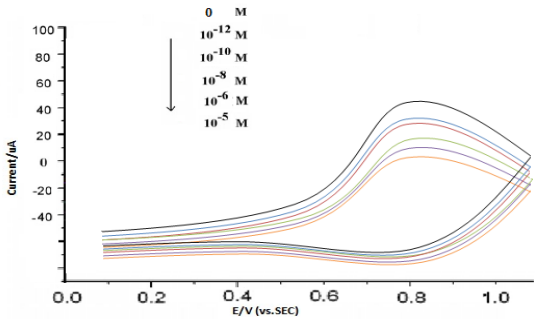

Figure 6: Electrochemical detection of pesticides

3.1.6. AChE reactivation

After exposure to pesticides, the AChE/CuO/G. electrode was firstly washed with 0.1 M pH 7 PBS, then reactivated by immersing in 3-10 Mm paraoxon for 12 min, and then transferred to 0.1 M pH 7 PBS containing 0/1 mM ATCh for CV analysis of the electrochemical response. The reactivation efficiency was calculated as follows:

R(%) =(Ir /IP, control).100%

where Ir was the peak current of 1 mM ATCh on AChE/CuO/G.E .

3.1.7. Stability of the paraoxon biosensor

The stability of AChE/CuONPS/G.E biosensor has been checked by carrying out experiments at the regular interval of a week and it has been found that AChE/CuO NPS/G.E based electrochemical biosensor retain its 92% 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 copper oxide nanoparticles on the gold electrode.

CONCLUSION

Gold electrode modified with nanoparticles of copper oxide was employed for the electro catalytic reduction and determination of paraoxon. Catalyses can be effectively immobilized on copper oxide nanoparticles modified gold electrodes to produce a fast direct electron transfer. The immobilized catalyzes maintain its bioactivity and native structure. The combination of catalyzes enzyme and cuo NPS in the biosensor would result in the improvement of analytical performance, characterized by broader linear range and lower detection limit for paraoxon determination.

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