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An amperometric biosensor based on acetylcholinesterase immobilized onto iron oxide nanoparticles/multi-walled carbon nanotubes modified gold electrode for measurement of organophosphorus insecticides

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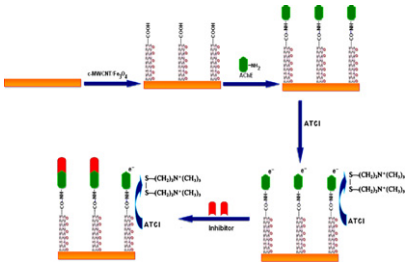
HIGHLIGHTS

- Constructed a novel composite material using Fe_3O_4NP and c-MWCNT at Au electrode for electrocatalysis.
- The properties of nanoparticles modified electrodes were studied by SEM, FTIR, CVs and EIS.
- The biosensor exhibited good sensitivity (0.475 mA μM^{-1})
- The half life of electrode was 2 months.
- The sensor was suitable for trace detection of OP pesticide residues in milk and water.

GRAPHICAL ABSTRACT

The stepwise amperometric biosensor fabrication process and immobilized acetylcholinesterase inhibition in pesticide solution.



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ABSTRACT

An acetylcholinesterase (AChE) purified from maize seedlings was immobilized covalently onto iron oxide nanoparticles (Fe_3O_4NP) and carboxylated multi walled carbon nanotubes (c-MWCNT) modified Au electrode. An organophosphorus (OP) biosensor was fabricated using this AChE/Fe_3O_4/c-MWCNT/Au electrode as a working electrode, Ag/AgCl as standard and Pt wire as an auxiliary electrode connected through a potentiostat. The biosensor was based on inhibition of AChE by OP compounds/insecticides. The properties of nanoparticles modified electrodes were studied by scanning electron microscopy (SEM), Fourier transform infrared (FTIR), cyclic voltammograms (CVs) and electrochemical impedance spectroscopy (EIS). The synergistic action of Fe_3O_4NP and c-MWCNT showed excellent electrocatalytic activity at low potential (+0.4 V). The optimum working conditions for the sensor were pH 7.5, 35 °C, 600 μM substrate concentration and 10 min for inhibition by pesticide. Under optimum conditions, the inhibition rates of OP pesticides were proportional to their concentrations in the range of 0.1–40 nM, 0.1–50 nM, 1–50 nM and 10–100 nM for malathion, chlorpyrifos, monocrotophos and endosulfan respectively. The detection limits were 0.1 nM for malathion and chlorpyrifos, 1 nM for monocrotophos and 10 nM for endosulfan. The biosensor exhibited good sensitivity (0.475 mA μM^{-1}), reusability (more than 50 times) and stability (2 months). The sensor was suitable for trace detection of OP pesticide residues in milk and water.

1. Introduction

Organophosphorus (OP) insecticides are widely used in agriculture. Their presence in water, food and animal feeds presents a potential hazard due to their high mammalian toxicity [1]. The most general approach for determination of the organophosphorus compounds is based on their property of inhibiting some important enzymes [2], e.g. acetylcholinesterase (AChE), which is essential for the functioning of the central nervous system but whose inactivity causes respiratory paralysis and death [3]. Hence, a rapid and reliable quantification of trace level of OP compounds is important for monitoring their potential hazard to health and the environment

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[4]. In view of the unavoidable routes of OPs into biological systems, different analytical methods and technologies have been used to determine them. Presently, the most common techniques are gas chromatography [5], high-performance liquid chromatography [6] and gas chromatography coupled with mass spectrometry [7]. Although these methods provide very good sensitivity and accuracy, these are currently limited to laboratory analysis, due to the requirements of sophisticated and expensive equipment, extensive time, highly trained personnel and complicated sample pretreatments [8]. Hence, rapid, sensitive and field deployable methods are still needed for simple, rapid and reliable detection of OP.

As a good alternative and with the improvement of being quick and consistent, biosensors based on inhibition of enzyme acetylcholinesterase and coupled with simple detectors were developed recently [9–16], which are considered a viable alternative to the chromatographic methods in pesticide determination for on-site analysis. Besides being specific and sensitive, they are portable, less expensive and do not require tedious sample pretreatment [17]. Various nanoparticles based enzyme (AChE) electrodes have been reported for determination of pesticides such as ZrO_2 NPs-modified screen printed electrode [18], multi-walled carbon nanotube modified GCE [19], AuNPs–polypyrrole nanowires composite film modified GCE [20], gold–platinum bimetallic nanoparticles onto 3-aminopropyltriethoxy silane modified GC electrode [21], Au-MWNTs-modified GC electrode [22], one-dimensional gold nanoparticles onto GC electrode [23] and prussian blue modified GC electrode [24]. However these electrodes suffer from leakage of enzyme resulting into low stability of enzyme electrode and require several operations involving pretreatment of the sample. In electrochemical studies, electrodes made up of commonly used solid materials, such as graphite, carbon paste and glassy carbon (GC), exhibit common disadvantages, e.g. the electrode area and the activity of the metal deposited electrically change continuously and oxide layers affecting the reproducibility of the measurements [25]. Covalent immobilization of enzyme not only overcomes this problem but also leads to better biomolecule activity and greater stability.

Carbon nanotubes (CNT) are the promising nanomaterials recently explored for chemical and biological sensing application. They are hollow graphitic cylinders with electrocatalytic effect and a fast electron-transfer rate [26–28]. Among a wide variety of metal oxide nanoparticles, Fe_3O_4 nanoparticles are particularly attractive due to their unique magnetic and electrical properties [29]. Nano-sized magnetic bioconjugated materials have been used in electrochemical biosensor devices due to many potentially unique properties such as large surface area, higher bioactivity, excellent conformation stability and better contact between biocatalyst and its substrate [30,31]. We describe herein a unique approach of immobilizing covalently maize acetylcholinesterase onto Fe_3O_4/c-MWCNT modified gold electrode (AuE) and its application in construction of an amperometric biosensor for determination of pesticides. Fe_3O_4/c-MWCNT nanocomposite based AChE biosensor is expected to offer high sensitivity, high biocompatibility, high charge transfer rate and good stability.

2. Experimental

2.1. Chemical and reagents

Acetylthiocholine chloride (ATCl), 2-pyridine aldoxime methiodide [2-PAM], Sephadex G-100 and DEAE-Sephacel from Sigma Chemical Co., USA. 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), $(NH_4)_2{\cdot}Fe(SO_4)_2{\cdot}6H_2O$ and $NH_4Fe(SO_4)_2{\cdot}6H_2O$ from Sisco Research Laboratory, Mumbai, carboxylated multi-walled carbon nanotubes (Functionalized MWCNT or c-MWCNT) (12 walls, length 15–30 μm, Purity 90%, Metal content: nil) from Intelligent Materials Pvt. Ltd., Panchkula (Haryana) India were used. The pesticides (malathion, chlorpyrifos, monocrotophos and endosulfan) were from Lentrek North America (San Francisco, CA). Au wire (1.5 cm × 0.05 cm) (23 carat) was from local market. All other chemicals were of analytical reagent (AR) grade. Double distilled water was used throughout the experiments.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) measurements were performed on a potentiostat/Galvanostat (Autolab, Eco Chemie, The Netherland. Model: AUT83785) with a three electrode system composed of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode and modified Au wire as a working electrode. Fourier transform infrared (FTIR) spectroscopy was performed in spectrometer (model iS10, Thermoelectron, USA). Ultrasonication was performed on Misonix Ultrasonic Liquid Processors (mode XL-2000 series). Scanning electron microscopy (SEM) measurements were carried out at Department of Chemistry, M.D. University, Rohtak. X-ray diffraction (XRD) studies of Fe_3O_4NP and c-MWCNT were carried out at Physics Department G. J. University, Hisar, using X-ray diffractometer (make: Rigaku Mini Flex II, Americas Corporation).

2.3. Purification of AChE from maize seedlings

The extraction and purification of enzyme was carried out in cold (4–10 °C). Two hundred grams of 5-day-old etiolated maize seedlings were homogenized in a 4-fold volume (w/v) of 10 mM potassium phosphate buffer (pH 7.0) containing 10 mM EDTA and 4% ammonium sulfate. The homogenate was left for 1 h in the dark, and then the supernatant was collected by centrifugation at 6000 × *g* for 15 min and stored at 4 °C. The pellet was resuspended in a 3-fold volume (w/v) of the extraction buffer, stirred for 1 h and recentrifuged for collection of supernatant. This procedure was repeated twice. The supernatants were combined and brought to 80% saturation with ammonium sulfate and left overnight. The resulting precipitates were collected by centrifugation at 15,000 × *g* at 4 °C for 15 min, resuspended in 3 mL of 10 mM phosphate-buffered saline, pH 7.4 and then dialyzed against the same buffer. The precipitate that formed during dialysis was removed by centrifugation and the supernatant was subjected to gel filtration on Sephadex G-100 column (1.4 cm × 23 cm) run in 0.05 M potassium phosphate buffer (pH 7.0) and ion exchange chromatography on DEAE-Sephacel column (2.5 cm × 12.5 cm) using a linear gradient of 0.1 M to 0.6 KCl in 0.02 M potassium phosphate buffer (pH 6.7) [32]. The active fractions were pooled and treated as purified enzyme.

2.3.1. Testing of purity of AChE

The purity of purified enzyme was tested by polyacrylamide gel electrophoresis (PAGE) using Coomassie blue as protein stain.

2.3.2. AChE assay

AChE preparation (100 μL) was added to 150 μL of distilled water. After pre-incubation at 30 °C for 10 min, 250 μL of 12.5 mM acetylthiocholine chloride in 100 mM sodium phosphate buffer, pH 7.0, was added to diluted enzyme and incubated again at same temperature (30 °C) for 120 min. After incubation, 300 μL of the reaction mixture was transferred to a new vial, and then 1425 μL of 100 mM sodium phosphate buffer, pH 7.0 and 75 μL of DTNB in 100 mM sodium phosphate, pH 7.0, were added to this vial. The A_{412} was read after 1 min. The control was run in the same manner as described above except that the enzyme was replaced by D.W [32].