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Simultaneous detection of monocrotophos and dichlorvos in orange samples using acetylcholinesterase–zinc oxide modified platinum electrode with linear regression calibration

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ABSTRACT

Monocrotophos (MCP) and dichlorvos (DDVP) belong to the group of organophosphate pesticides and are categorized as highly toxic by the World Health Organization. Consumption of oranges containing MCP and DDVP leads to harmful health effects such as DNA damage, apoptosis, cardiotoxicity and cancer. It also causes chronic disorders such as eye irritation, miosis, blurred vision; dizziness, convulsions, dyspnea salivation, abdominal cramps, nausea, diarrhea and vomiting. In this context, an acetylcholinesterase modified Pt working electrode for the simultaneous detection of MCP and DDVP in orange samples was fabricated using zinc oxide nanospheres as a nano-interface. An analytical method for the simultaneous detection of MCP and DDVP was constructed by establishing 24 linear regression models and error analysis was performed to verify the practicability of these models. The developed biosensor achieved a detection limit of MCP and DDVP with detection limits of 0.036 and 0.012 nM respectively. AChE enzyme was successfully reactivated using 2-pyridine aldoxime methiodide. The developed biosensor exhibited good recovery, thus providing a promising tool for analysis of MCP and DDVP.

1. Introduction

Oranges are consumed in a large number throughout the world for their richness in vitamin C [1]. India is the fourth largest producer of oranges in the world. A national report revealed that monocrotophos (MCP) and dichlorvos (DDVP) were the predominantly used pesticides for controlling pests in oranges. The Maximum Residue Limits (MRL) for MCP and DDVP in orange are 0.2 mg/kg and 0.1 mg/kg respectively. MCP and DDVP belong to the vast group of organophosphorus pesticides [2] and are being used widely in agriculture due to their high efficiency and availability [3]. The regional quarantine center in Faizabad and Chennai found out that these pesticide residues were exceeding the MRL. Forensic reports of the food samples in the Bihar (India) mid-day meal tragedy (16th of July, 2013) revealed that MCP was the contributor to the death of 23 school children [4]. MCP and DDVP are declared as highly hazardous by the World Health Organization [5,6]. They are carcinogenic and also cause several neurotoxic disorders. The use of increased concentrations of MCP and DDVP can lead to destruction of life [7,8]. Hence the detection of these pesticides is central.

In literature, several techniques have been previously reported for the detection of MCP and DDVP pesticides. Liquid chromatography and mass chromatography coupled with mass spectroscopy are the techniques conventionally used for the detection of these pesticides [9,10]. Although these methods produce efficient results, they are highly time-consuming and require well trained technicians to perform the analysis [11]. Even though, many biosensors are reported for the detection of MCP and DDVP using acetylcholinesterase (AChE) as enzyme, it is essential for designing a biosensor that can simultaneously detect both MCP and DDVP. Inhibition based electrochemical biosensors being user friendly, rapid and easy to fabricate, are preferred over other techniques [11].

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Since organophosphorus pesticides can inhibit AChE, AChE enzyme is chosen by many researchers for the simultaneous detection of monocrotophos and dichlorvos pesticides. Acetylcholinesterase catalyzes the hydrolysis of acetylthiocholine chloride (ATChCl) to thiocholine (oxidized) [12].

$$\text{Acetylthiocholinechloride} + H_2O \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Aceticacid} + Cl^-$$

Organophosphorus pesticides phosphorylate the serine in the active centers and inhibit this reaction [13]. Hence the immobilization of AChE with the electrode proves to be advantageous towards the detection process in the electrochemical sensor. Addition of a nanointerface along with the AChE matrix enhances the electron transfer, sensitivity and stability [13]. Previously Dimcheva et al., [14] have used AChE immobilized on gold nanoparticles for MCP detection and achieved a linear range of 50–400 nM and detection limit of 1 μM. In another case, Wei and Wang [15] have fabricated an electrochemical sensor for DDVP detection using gold nanoparticles–porous carbon and obtained linear range of 4.5×10^{-4} nM–4.5 nM and detection limit of 2.99×10^{-4} nM.

In this work, platinum (Pt) working electrode was modified using zinc oxide (ZnO) nano-interface and AChE matrix. ZnO nano-interface is preferred due to the high isoelectric point (pI = 9.4) when compared to the AChE enzyme (pI = 6.6) and hence enhances the immobilization and electrical conductivity [12]. Zinc oxide (ZnO) nanoparticles were synthesized by solvothermal method. X-Ray Diffraction (XRD) and Field Emission Scanning Electron Microscopy (FE-SEM) studies were carried out to determine structural and morphological characteristics respectively. AChE was immobilized by physical entrapment method and the immobilization of the AChE enzyme on the surface of ZnO nano-interface was confirmed through Fourier Transform-Infrared (FTIR) spectroscopy. Several parameters such as the pH, AChE enzyme amount, scan rate, ATChCl concentration, permeability, incubation were optimized. AChE inhibition by the binary mixture of MCP and DDVP was observed first and then the influence of electrochemical parameters on the binary mixture of MCP and DDVP was analyzed through principle component analysis.

2. Materials and methods

2.1. Materials

2-Pyridine aldoximemethiodide, MCP, DDVP, zinc acetate dihydride, AChE (Type C- 3389, 500 UN from electric eel) and acetylthiocholine chloride (ATChCl) of molecular weight 197.73 g mol^{-1} were purchased from Sigma–Aldrich, USA. Sodium hydroxide, potassium hydroxide, glucose, lactic acid, chitosan (molecular weight: 140.000 g mol^{-1}), acetic acid, ascorbic acid, monobasic sodium phosphate monohydrate and dibasic sodium phosphate dehydrate were obtained from Merck India Ltd., India. Cadmium acetate dehydrate was purchased from Loba Chemie Pvt., Ltd., India. Urea, nickel chloride and cupric acetate were procured from Thermo Fisher Scientific Pvt., Ltd., India. Platinum wire counter electrode (CHI115, 0.5 mm diameter), 0.4 M KCl saturated Ag/AgCl reference electrode (CHI111, 0.5 mm diameter) and Pt working electrode (CHI102, 2 mm diameter) were obtained from CH Instruments, Inc., USA. All the solutions were prepared using deionized water (AQUA Purifications Systems, India).

2.2. Synthesis of ZnO nanoparticles

0.1 M of zinc acetate dihydrate solution was prepared in 100 mL of deionized water. In addition, 2.0 M of KOH was prepared and added drop wise to the zinc acetate dihydrate solution under continuous stirring. A white jelly like precipitate was obtained. Then, the obtained precipitate was heated and maintained at 353 K for 3 h. The resulting precipitate was centrifuged and washed with distilled water. Then it was dried overnight at 343 K. The suspension was then annealed at 773 K for 6 h.

2.3. Fabrication of modified electrode

ZnO nanoparticles were sonicated with 100 μL of PBS and 10 μL of chitosan for 20 min. Then 10 μL of AChE was added to the prepared solution and sonicated for 1 min. The Pt electrode was polished with alumina powder. Then 3 μL of the resulting mixture was immobilized on the Pt electrode. Finally, the modified Pt/ZnO/AChE/Chitosan electrode was washed and dried.

2.4. Characterization studies

The morphologies of ZnO, ZnO/AChE and ZnO/AChE/Chitosan nanoparticles were observed using Field Emission Scanning Electron Microscope (FE-SEM) (Model JSM 6701F, JEOL, Japan) and Field Emission Transmission Electron Microscope (FE-TEM, Model JSM 2100F JEOL, Japan). Image J 1.48 q software was used to calculate the grain size of ZnO nanoparticles. The structure of ZnO nanoparticles was observed using X-ray Diffractometer (XRD) (Rigaku Ultima III, USA) with Cu Kα radiation of wavelength 1.5408×10^{-10} m. Electrochemical studies were performed using electrochemical workstation (CHI600C, CH Instruments, USA) with the Pt/ZnO/AChE/Chitosan as working electrodes, Ag/AgCl saturated with 0.1 M KCl as a reference electrode and Pt wire as a counter electrode. All electrochemical measurements were carried out at room temperature in PBS at a pH of 8.0.

2.5. AChE inhibition

The cyclic voltammogram of Pt/ZnO/AChE/Chitosan bioelectrode was measured in 0.1 M PBS (pH 8) containing 0.9 mM ATChCl, and then the Pt/ZnO/AChE/Chitosan electrode was incubated with different concentrations of MCP and DDVP for 25 min. The AChE activity in the modified electrode was analyzed before and after the exposure of MCP and DDVP. The percentage AChE inhibition is calculated using,

$$I\% = \frac{J_o - J_i}{J_o} \times 100$$

where J_i and J_o represent the current density of ATChCl on Pt/ZnO/AChE/Chitosan with and without MCP and DDVP inhibition, respectively.

2.6. AChE enzyme reactivation

After exposure of Pt/ZnO/AChE/Chitosan to monocrotophos and dichorvos, it was washed with 0.1 M PBS (pH 8.0) and reactivated with 4.0 mM 2-pyridine aldoximemethiodide [2-PAM] for 30 min, then Pt/ZnO/AChE electrode was exposed to 0.9 mM ATChCl to study the electrochemical response. The reactivation efficiency (R(%)) was estimated using,

$$R\% = \frac{J_r - J_{\text{p.exp}}}{J_{\text{p.control}} - J_{\text{p.exp}}} \times 100$$

where J_r is the current density of ATChCl on Pt/ZnO/AChE/Chitosan with 2-PAM reactivation, $J_{\text{p.exp}}$ is the current density of ATChCl on Pt/ZnO/AChE with MCP and DDVP inhibition and $J_{\text{p.control}}$ is the current density of ATChCl on Pt/ZnO/AChE/Chitosan electrode with 2-PAM reactivation.