Journal of Electroanalytical Chemistry 823 (2018) 617–623

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Magnetite nanoparticles/chitosan-modified glassy carbon electrode for non-enzymatic detection of the endocrine disruptor parathion by cathodic square-wave voltammetry

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ARTICLE INFO

Keywords:
Glassy carbon electrode
Magnetite nanoparticles
Chitosan
Parathion
Food samples

ABSTRACT

A glassy carbon electrode modified with magnetite nanoparticles dispersed in chitosan was employed as electrochemical detector for the determination of parathion in food samples by cathodic square-wave voltammetry. The modified electrode surface was characterized by scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. Under optimized experimental conditions, the square-wave voltammograms for parathion obtained with the proposed modified electrode exhibited a well-defined peak at −0.59 V, corresponding to the reduction of the nitro group to the hydroxylamine group. The resultant cathodic peak current was linear for parathion concentrations in the range of 0.34 to 4.46 μmol L^{-1} (r = 0.997). The limits of detection and quantification were 0.187 and 0.567 μmol L^{-1}, respectively. The novel detector was successfully employed for the analysis of parathion in five food samples (carrot, orange, tomato, lettuce and rice) using the standard addition method. The results were statistically compared with data obtained using the UV–vis technique.

1. Introduction

Organophosphate pesticides (OPs) are highly toxic agricultural chemicals that are extensively used worldwide to control a wide variety of insect species. A notable OP is parathion (*O*,*O*-diethyl-*O*-4-nitrophenylthiophosphate, Fig. S1). It is one of the most commonly used insecticides, emerging as an alternative to DDT and other chlorinated hydrocarbon pesticides [1]. However, the increasing production and application of parathion for use in agriculture has resulted in the contamination of fruits, vegetables, water resources and soil. This is potentially harmful and poses a serious risk because parathion can act as a lethal poison when ingested by humans and animals [2–4]. The acute toxicity of OPs such as parathion results from the irreversible inhibition of acetylcholinesterase (AChE). This enzyme is a serine hydrolase responsible for the breakdown of the neurotransmitter acetylcholine at neuronal synapses and neuromuscular junctions. The inhibition of AChE leads to an accumulation of acetylcholine, resulting in hyperstimulation of the cholinergic system, paralysis, seizures and respiratory failure, leading to death [3, 5, 6]. Even at very low concentrations, continuous exposure to OPs can cause headaches, dizziness, nausea, decreased heart beat and fever [7]. OPs are classified as endocrine disruptors (EDs) which, according to the U.S. Environmental Protection Agency (EPA), are exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones present in the body, which are responsible for homeostasis, reproduction, and developmental processes [7, 8]. Because of the high toxicity of OPs, the rapid and sensitive detection of these compounds is extremely important for environmental security and health protection.

The traditional methods for the detection of OPs include high performance liquid chromatography (HPLC) [9, 10], gas chromatography (GC) [11], chromatography-mass spectrometry [12, 13], enzyme-linked immunosorbent assay (ELISA) [14, 15] and electrochemical sensing [16–18]. Notably, electrochemical sensing is gaining much attention because it offers the advantages of low-cost, ease of sample preparation, portability of equipment and, most importantly, high sensitivity, which allows it to be employed in the quantification of pesticides at trace-levels and in routine analysis [4, 19]. The high practical relevance of developing electrochemical sensing devices for OPs has motivated researchers to focus on different analysis strategies. These include the use

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https://doi.org/10.1016/j.jelechem.2018.07.007
Received 27 February 2018; Received in revised form 3 July 2018; Accepted 3 July 2018
Available online 09 July 2018
1572-6657/

of chemically modified electrodes (CME), which has been extensively explored [20]. In this field, several inhibition and non-inhibition biosensor systems, based on the immobilization of acetylcholinesterase or organophosphate hydrolase onto various electrochemical transducers, have been reported [20–25]. In addition to enzymes, nanoparticles (NPs) of different materials are also widely used in the preparation of CMEs for OP detection, due to their high surface to volume ratio and particular electrical and catalytic properties [20]. In recent years, magnetic nanoparticles have been attracting much interest for the development of electrochemical devices because of their unique multifunctional properties, such as small size, good biocompatibility, superparamagnetism, low toxicity, ease of preparation, high adsorption ability, low cost and convenient surface functionalization [26–28]. However, the tendency of these NPs to aggregate makes it necessary to disperse them in a matrix capable of improving the stability. Thus, stabilizers such as surfactants and polymeric compounds with some specific functional groups have been used to stabilize and/or functionalize the surface of the nanoparticles [29]. Chitosan (CS) is a polyaminosaccharide with unique biological and chemical properties. It has been widely used in the fields of medicine, medicaments and biotechnology [27]. Due to the presence of the reactive amino and hydroxyl functional groups, CS is one of the most promising immobilization matrices and it is commonly used to enhance the stability of nanoparticles of silver [30], gold [31], iron and iron oxide [27], platinum and palladium [32], among others. In addition, CS offers excellent membrane-forming ability, good adhesion, low cost, non-toxicity, high mechanical strength and hydrophilicity [33]. Therefore, because of its desirable properties, CS has been combined with magnetite (iron (II, III) oxide - Fe_3O_4) NPs in electrochemical sensing platforms [27].

In this study, a glassy carbon electrode surface modified with Fe_3O_4 NPs nanoparticles and a CS film (CS-Fe_3O_4/GCE) was prepared and characterized. The electrochemical behavior and detection of parathion at this modified electrode was studied. Despite the use of parathion be banned, it is still used by farmers due to lack of supervision. In this sense, the development of devices for its quantification is extremely important considering its high toxicity. The experimental conditions were optimized and the detection of parathion in food samples was carried out employing cathodic square-wave voltammetry (CSWV). The voltammetry results were compared with data obtained by UV–vis spectroscopy to evaluate the performance of the proposed CS-Fe_3O_4/GCE detector.

2. Material and methods

2.1. Reagents, solutions and samples

All reagents employed in this study were of analytical grade and purchased from Sigma-Aldrich. They were used without further purification. The CS was of low molecular weight. Fe_3O_4 NPs were used to modify the electrode. The main characteristics of magnetite nanoparticles informed by the manufacturer (Sigma Aldrich – Product Number 637106-25G – Batch Number MKBR5062V) are: product characterized by TEM, XRD and ICP, black powder, spherical, diameter smaller than 50 nm, 97% purity (based on trace metals analysis), BET surface area higher than 60 $m^2\,g^{-1}$, and bulk density of 0.84 $g\,mL^{-1}$. Aqueous solutions were prepared using ultrapure water with a resistivity of 18.2 MΩ cm obtained from a Milli-Q system (Millipore, USA). A stock solution of parathion was prepared at a concentration of 35.0 $mmol\,L^{-1}$ in ethanol and kept in a refrigerator. Working solutions were freshly prepared before use through the dilution of the stock solution. Three buffer solutions (0.2 $mol\,L^{-1}$) were tested as the supporting electrolyte: Britton-Robinson (B-R) (H_3BO_3/CH_3COOH/H_3PO_4), phosphate (H_3PO_4/$NaH_2PO_4{\cdot}H_2O$) and McIlvaine (Na_2HPO_4/$C_6H_8O_7$). The buffer solutions were kept at 5 °C for around 90 days. Their pH was adjusted before use with 6.0 $mol\,L^{-1}$ HCl or NaOH solutions.

Five food samples were used to evaluate the effect of the matrix on the parathion detection: lettuce, tomato, carrot, orange and rice. The preparation was carried out by grinding 20.0 g of the fresh sample, which was spiked with 15.0 $\mu g\,g^{-1}$ of parathion. The extraction of parathion from each sample was then carried out with 50.0 mL of ethanol/water solution (50:50, v/v) for 60 min at 70 °C. In the next step, the mixture was subjected to ultrasound for a further 60 min. Finally, the ethanolic extracts were filtered using a filter paper of medium porosity (25 μm) and stored at 5 °C for 48 h. For the detection of parathion, 500 μL of the filtered ethanolic extracts was added to the electrochemical cell containing the supporting electrolyte.

2.2. Preparation and characterization of CS-Fe_3O_4/GCE detector

CS was dissolved in 0.1 $mol\,L^{-1}$ B-R buffer to form a 0.25% (w/w) solution and then filtered through filter paper (25 μm). The pH value was adjusted to 7.0 with a 6.0 $mol\,L^{-1}$ NaOH solution. The CS-Fe_3O_4 mixture was obtained by dispersing different amounts of Fe_3O_4 NPs in 5.0 mL of the CS solution. The suspension was mixed using ultrasonic irradiation for 10 min. Lastly, a viscous solution of CS with uniformly dispersed Fe_3O_4 NPs was obtained.

The drop-coating method was used to prepare the CS-Fe_3O_4/GCE. Prior to modification, the bare GCE was polished with 0.05 μm alumina powder, then rinsed with ultrapure water and dried in air. In the next step, 5.0 μL of CS-Fe_3O_4 suspension was dropped onto the GCE surface. The solvent was then evaporated under vacuum for 10 min to make the modified electrode CS-Fe_3O_4/GCE. For comparison purposes, a CS/GCE was also obtained by dropping CS on the GCE surface. The morphology of the CS-Fe_3O_4/GCE surface was analyzed by scanning electron microscopy with field emission gun (SEM-FEG) using a JEOL JSM-6701F microscope (JEOL, Japan). For the SEM-FEG analysis, 10.0 μL of the CS-Fe_3O_4 suspension was dropped onto a glassy carbon plate (area = 1.0 cm^2, thickness = 1 mm) and dried under vacuum. After, the glassy carbon plate was washed with distilled water and kept under vacuum for 24 h. The electrochemical characterization of the proposed modified electrode was performed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using a PalmSens3 potentiostat (Palm Instruments BV, Netherlands). The CV measurements were recorded in a 0.1 $mol\,L^{-1}$ KCl solution containing 1.0 $mmol\,L^{-1}$ $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ (1:1) as redox probe, in the potential range of −0.3 to +0.8 V at a scan rate of 10 to 300 $mV\,s^{-1}$. EIS spectra were obtained in 0.1 $mol\,L^{-1}$KCl containing 5.0 $mmol\,L^{-1}$ $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ (1:1) at the open circuit potential, using an amplitude of 5 mV and a frequency range of 0.1 to 50,000 Hz.

2.3. Electrochemical measurements

CV and CSWV measurements were carried out with a portable PalmSens3 potentiostat (Palm Instruments BV, Netherlands) coupled to a computer running the PSTrace software (version 4.8).

A three-electrode electrochemical cell (15 mL) was used with the GCE or the modified GCE as the working electrode, an Ag/AgCl electrode (saturated with KCl) as the reference electrode and a Pt wire as the auxiliary electrode. Electrochemical measurements (CV and CSWV) were carried out in 10 mL of buffer solution (B-R, phosphate or McIlvaine). All pH measurements were taken using a pH meter, model HI 2221 (HANNA Instruments, USA). The CV measurements were recorded by cycling the potential between 0.0 and −1.0 V at a scan rate of 50 $mV\,s^{-1}$. The parameters of the SWV (ΔE_s - potential increment, a - amplitude and f - frequency) were optimized in the following ranges: ΔE_s = 1 to 10 mV, a = 10 to 100 mV and f = 10 to 100 Hz. For the construction of the calibration curve, the CSWV measurements were performed sweeping the potential from 0.0 to −1.0 V at f = 40 Hz, a = 40 mV and ΔE_s = 4 mV, after successive additions of a stock solution of parathion. For the standard addition method employed in the