Journal of Electroanalytical Chemistry 775 (2016) 121–128

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Conducting polymer and multi-walled carbon nanotubes nanocomposites based amperometric biosensor for detection of organophosphate

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 21 May 2016
Accepted 23 May 2016
Available online 24 May 2016

Keywords:
PEDOT-MWCNTs
Organophosphates
Acetylcholinesterase
Bioelectrode
Spiked sample

ABSTRACT

A nanocomposite consisting of conducting polymer (CP)- Poly(3,4-ethylenedioxythiophene) (PEDOT) and multi-walled carbon nanotubes (MWCNTs) has been deposited electrochemically onto the surface of fluorine doped tin oxide (FTO) sheets for the analysis of malathion organophosphate (OP). The –COOH functionalization of MWCNTs has been done for the covalent immobilization of an enzyme acetylcholinesterase (AChE). The prepared PEDOT-MWCNTs/FTO and AChE/PEDOT-MWCNTs/FTO bioelectrodes were characterized by Fourier transform infrared spectrometry (FTIR), Field emission-scanning electron microscopy (FE-SEM) and electrochemical studies. Various optimization studies were done for different parameters including pH of 0.1 M phosphate buffer solution (PBS) (7.5), AChE concentration (50 mU), substrate concentration (0.3 mM) and inhibition time (10 min). The detection limit for malathion was calculated to be 1 fM within the linear range 1 fM to 1 μM. The inhibited AChE could be regenerated to 99% by 2-PAM. The storage stability and reusability of the prepared bioelectrode is observed to be 30 days and seven times, respectively. Recoveries of malathion from the spiked lettuce sample ranged between 96–98% using the developed bioelectrode.

1. Introduction

Amperometric enzymatic acetylcholinesterase (AChE) biosensors fabricated from different nanoparticles emerged as valuable tools in the field of organophosphates (OPs) detection. Electrochemical biosensors possess well known advantages such as rapid response, low detection limit, high sensitivity, versatility and good reproducible results as compared to the traditional analytical techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with various detectors, capillary electrophoresis and spectroscopy [1–8]. The malathion OP acts as an irreversible inhibitor of AChE and when the food (apple, cabbages, lettuce etc.) containing malathion is being consumed, the metabolite- malaoxon is produced in the body which is more toxic and a strong inhibitor of AChE as compared to malathion. Therefore, detection of malathion is of utmost importance before it leads to the formation of such toxic compounds [8–10].

Immobilization of AChE enzyme onto a suitable matrix is a crucial step in the fabrication of an electrochemical enzymatic biosensor. Many electrode materials such as sol-gel, metal oxide NPs, conducting polymers (CPs), self-assembled monolayers (SAMs) etc. have been used to develop AChE inhibition based biosensors [11,12]. Among the use of various CP alike polyaniline (PANI), polythiophene, polypyrrole (PPy) etc. Poly(3,4-ethylenedioxythiophene) (PEDOT) has recently sparked much interest in the research field due to its evidently superior qualities such as high stability, enhanced light transmission, processibility and simplicity of production over other polymers. Conductivity in PEDOT is a result of conjugated backbone with high degree of π orbital overlap. Yamata et al. 1995 reported that PEDOT films retained 89% of their electrochemical activity after polarization for 16 h in solution with pH 7.5, while only 5% found in polypyrrole. The similar results were obtained with low pH 6 [13]. This unique property contributes toward the long life of biosensor in comparison to other CPs based sensors. PEDOT is a highly conductive polymer that can be polymerized by either oxidative chemical polymerization, organic chemical vapor deposition or electrochemical polymerization. PEDOT has been used for development of various biosensor and electronic tongues with improved characteristics in comparison to other CPs and metal electrodes. Because of the high electronic and ionic conductivity, thermal and chemical stability, PEDOT as compared to other polymers (PANI, PPy etc.) can be used as a mediator in biosensor [14,15]. It is evident that conductivity, selectivity, processability, low redox potential and stability are the most appealing properties provided by PEDOT and they serve as a driving force for further development in the area of electrochemical sensors.

CP along with the carbon materials are extensively used to combine the properties of the individual components for a synergistic performance in biosensor fabrication. Carbon nanotubes (CNTs) have the

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http://dx.doi.org/10.1016/j.jelechem.2016.05.037
1572-6657/

ability to provide high external surface area and promote electron transfer, high sensitivity, low limit of detection, chemical stability, biocompatibility and non-toxicity [16–19]. CNTs have been widely used in biosensor designs and nanoscale electronic devices due to their favorable microenvironment around enzyme or other biomolecules. Chemically modified CNTs are mainly merged into a polymeric matrix. Carboxylation of the CNTs with sulphuric and nitric acid makes them more soluble in polar solvents and provides functional groups for the immobilization of biomolecules [20,21]. Polythiophene doped MWCNTs modified glass carbon electrode (GCE) is being employed for the development of glucose biosensor [22]. MWCNTs linked to the nanoporous gold electrode through self-assembly technique along with the immobilized AChE enzyme are used for the measurement of OPs [10]. The common methods for the biomolecule immobilization on the surface of prepared nanomaterials include physical adsorption, covalent, cross-linking and entrapment [8,23].

PEDOT/MWCNTs materials leads to the formation of new nanocomposite with enhanced electrical and mechanical properties, mechanical stability, fast electron transfer rates and good sensitivity. Electropolymerization of PEDOT/MWCNTs on the surface of substrate controls the film morphology, thickness and electrical conductivity. Electrodeposited PEDOT-MWCNTs has been used for the detection of various analytes such as dopamine, hydroquinone and improvement of neural interface in bioengineering [24–26]. However, there is no evidence regarding the use of PEDOT-MWCNTs for detection and estimation of OPs till date.

The studies revealed that electroactive properties of PEDOT and MWCNTs enhanced overall performance of the bioelectrode for target analyte analysis. A simple, stable, effective and low-cost process has been developed in which AChE was covalently linked and stabilized on the surface of PEDOT-MWCNTs/FTO electrode for the amperometric analysis of OP using malathion as a model compound.

2. Experimental

2.1. Materials

AChE (Type C3389, 500 U mg^{-1} from electric eel), acetylthiocholine chloride (ATCl), malathion, 3,4-ethylenedioxythiophene (EDOT), 2-pyridine aldoxime methiodide (2-PAM), sulphuric acid, nitric acid, 1-ethyl-3 (3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma–Aldrich Laboratories, Mumbai, India and used as received. Acetonitrile and Lithium perchlorate ($LiClO_4$) was received from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other reagents were of analytical grade. Deionized water was used throughout the experiment.

2.2. Instrumentation

All electrochemical measurements were performed on Galvanostat (Metrohm, Autolab Instruments, Netherland) in a three electrode cell configuration consisting of a platinum wire as the counter electrode, silver wire as a reference electrode and the AChE/PEDOT-MWCNTs/FTO bioelectrode as a working electrode. The prepared bioelectrode was chemically and morphologically characterized by Fourier transform infrared spectrometry (FTIR, Nicolet IS50 Thermo scientific) and Field emission- scanning electron microscopy (FE-SEM, Hitachi SU 8010).

2.3. Preparation of functionalized MWCNTs

MWCNTs were synthesized by catalytic chemical vapor deposition technique [27]. 10 mg of MWCNTs were dispersed and treated with 25 mL of 1:3 concentrated HNO_3 and H_2SO_4 solution for 2 h under ice bath sonication. The solution was kept at 20 °C for overnight and were washed with water and separated by centrifugation at 3000 rpm (793 rcf) for five times. The functionalized MWCNTs were dried at 60 °C. Further, they were immersed in a mixture consisting of 5 mg mL^{-1} EDC and 5 mg mL^{-1} NHS for 2 h at room temperature for the activation of carboxylic groups. Finally, the functionalized MWCNTs were collected and dried at 60 °C.

2.4. Electropolymerization of PEDOT-MWCNTs

Electrochemical polymerization of PEDOT-MWCNTs was performed in an aqueous solution of deionized water and acetonitrile (3:2) containing 1 mg MWCNTs, 50 mM EDOT and 0.1 M $LiClO_4$. The solution was mixed properly for the perfect polymerization of PEDOT-MWCNTs on fluorine doped tin oxide (FTO) sheets. Electrochemical polymerization of PEDOT-MWCNTs on FTO was accomplished at a constant potential of 1 V for 180 s by chrono-amperometry method using electrochemical workstation. The thickness of the film (2 μm) and electrical conductivity ($4.6 \times 10^{-7}\ \Omega^{-1}\ cm^{-1}$) was calculated according to the following (1) and (2) equations [28,29]:

$$d = QM/2F\rho \quad (1)$$

where d is thickness of the film, Q is specific area overall charge for electrodeposition,

M is molar mass of the monomer, F is Faraday constant and ρ is density of polymer.

Electrical conductivity (σ) is being determined as:

$$\sigma = d/AR \quad (2)$$

where d is thickness of the film, A is area of electrode and R is the Bulk resistance.

2.5. Preparation of AChE/PEDOT-MWCNTs/FTO bioelectrode and characterization studies

The working bioelectrode was prepared by dropping AChE enzyme solution (50 mU) onto the surface of PEDOT-MWCNTs/FTO and incubated at 4 °C for overnight in humid chamber. The bioelectrode was washed with water to remove the loosely adsorbed enzyme and stored at 4 °C when not in use. Scheme 1 illustrates the schematic representation of the enzymatic bioelectrode construction and performance. The hybrid PEDOT-MWCNTs nanocomposites are linked to each other through the Van der waals interactions. The COOH group introduced on an external surface of the MWCNTs binds to the amino group on the surface of AChE enzyme through covalent linkage. FTIR characterization studies have been carried out using KBr discs to confirm the successful immobilization of AChE. Surface morphological studies were analyzed using FE-SEM. Electrochemical characterizations like Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS) measurements were also done to affirm the successful preparation of bioelectrodes.

2.6. Optimization of parameters

Certain parameters such as pH of phosphate buffer solution (PBS), concentration of AChE and ATCl were optimized electrochemically in a solution containing 0.1 M PBS (pH 7.5) and 0.1 M KCl. Michaelis–Menten constant (K_m) of the free and immobilized enzyme was then calculated following the Lineweaver–Burk Eq.Eq. (3):

$$1/I_s = (K_m/I_{max})\,(1/C) + 1/I_{max} \quad (3)$$

where I_s is the steady-state current after the addition of substrate ATCl, I_{max} is the maximum current obtained under saturated substrate conditions and C is the bulk concentration of substrate. The K_m and I_{max} value was determined by analysis of the slope and intercept for the plot of reciprocals of the steady-state current versus ATCl concentration.