Journal of Molecular Liquids 198 (2014) 181–186

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Mobilized lipase enzymatic biosensor for the determination of Chlorfenvinphos and Malathion in contaminated water samples: A voltammetric study

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ARTICLE INFO

Article history:
Received 12 July 2013
Received in revised form 5 June 2014
Accepted 18 June 2014
Available online 2 July 2014

Keywords:
Lipase enzyme
Voltammetric method
Malathion
Chlorfenvinphos
p-Nitrophenyl Acetate

ABSTRACT

Concentration levels of organophosphorus pesticides like Chlorfenvinphos and Malathion were determined by lipase enzyme inhibition method. The developed enzymatic method was purely based on in situ generation of *p*-Nitrophenol by enzymatic hydrolysis of *p*-Nitrophenyl Acetate. The production of this in situ generated *p*-Nitrophenol concentration was depending on the effective hydrolysis of *p*-Nitrophenyl Acetate by the lipase enzyme. This electrochemically active *p*-Nitrophenol gave an anodic oxidation peak at a potential of +0.024 V versus SCE. The various parameters such as effective hydrolysis of substrate, substrate concentration and effect of pH were studied for lipase enzyme. A linear calibration for the Chlorfenvinphos and Malathion was obtained in the concentration ranges of 100–900 μM and 100–900 mM respectively with a correlation coefficient of 0.9894 and 0.9680 under the optimized conditions by following the incubation time of 25 min. The electrochemical experiments were performed in 0.1 M phosphate buffer solution (pH 7.0) at room temperature. The limit of detection and limit of quantification values were found to be 84.45 μM, 253.03 mM and 281.5 μM, 843.1 mM respectively for Chlorfenvinphos and Malathion under optimized conditions. The developed liquid state lipase enzyme sensor could be applied for the analysis of water samples contaminated with pesticides. The developed method gives the information about the intensity of toxicity of pesticides.

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1. Introduction

Different types of organophosphorus compounds and their related pesticides are considered to be toxic. They are widely used in agricultural practices as pesticides and insecticides for controlling of broad spectrum of pests and insects by contact, stomach and respiratory action [1]. The high toxicity of organophosphorus neurotoxins and their large use in modern agriculture practices have increased the public concerns, health risks and the consequent contamination of water and food sources [2]. However, their wide use has resulted in their widespread distribution in the environment which in turn shows adverse health implications, even at trace levels in both ground and surface water. The same type of compounds has been produced as possible nerve poisons, a further area of application is in the military [3]. Among the existing organophosphorus compounds the Chlorfenvinphos and Malathion pesticides (Table. 1) are most commonly used in the agriculture for the control of various types of pests. The widespread global use of pesticides for crop protection and other household uses has placed a great threat to human health and environment. The problem is steadily growing and becoming more serious, especially, in the developing countries.

Many efforts have been made in finding of the pesticides. In the last decades organochlorine insecticides (e.g. lindane, aldrin, and DDT) were progressively replaced by organophosphorus (e.g., Dibrom, Chlorfenvinphos and Malathion) and the derivatives of carbamic acid (e.g. aldicarb and carbaryl) insecticides, that show low persistence in the environment but represent a serious risk because of their high acute toxicity. In fact, they are inhibitors of the cholinesterase enzymes [4–6] involved in muscle contraction and impulse transmission in the nervous system. The development of efficient analytical methods for their estimation in food and environmental samples is of the present importance.

These small amounts of pesticide residues when present in water, food and animal feeds create a potential hazard due to their high mammalian toxicity. Hence, a rapid and reliable quantification of trace levels of organophosphorus compounds is essential for monitoring of their potential hazard to health and the environment [7]. In view of the unavoidable routes of organophosphorus compounds into biological systems, different traditional analytical methods and technologies have been used for their determination such as gas and liquid chromatography [8], gas-chromatography with mass spectrometry detection [9,10], liquid–solid extraction followed by liquid chromatography-diode array

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http://dx.doi.org/10.1016/j.molliq.2014.06.019
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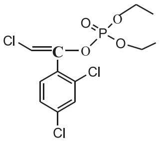
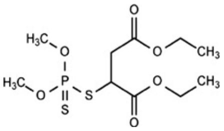
Table 1
The structure and molecular formulae of Chlorfenvinphos and Malathion.

Sl. No	Selected organophosphorus pesticide	Molecular formula	Chemical structure
01	Chlorfenvinphos,	$C_{12}H_{14}Cl_3O_4P$	
02	Malathion	$C_{10}H_{19}O_6PS_2$	

detection [11]. However, these methods can have very low limits of detection (LOD) and they are highly selective and sensitive. These methods are not applicable for field determinations, time-consuming and require sophisticated laboratory equipments [12].

Electroanalytical (voltammetry) methods have shown remarkable advantages in the analysis of environmental samples which are contaminated by toxic metals, pesticide residues, synthetic bioactive molecules and also industrial effluents. In this context various types of modified working electrodes play a major role since three decades. A polyvinyl chloride (PVC) matrix based neutral carrier porphyrin (A and B) ionophores Cu^{2+} selective sensor, potentiometric sensor and copper (II) selective sensor based on dimethyl 4, 4(O-phenylene), bis (3-thioallophanate) in PVC matrix have been fabricated and were applied for the determination of copper in synthetic and real samples [13–15]. Similarly mercury, cobalt, chromium, lead and cadmium are equally important toxic elements in the environment, thus the determination of their levels in the environment by the applications of some of the developed ion selective electrodes in which highly selective mercury electrode with diamine donor ligand [16], mercury selective potentiometric sensor [17] and neutral carriers based polymeric membrane electrode for the selective determination of mercury (II) [18] were used. PVC membrane based cadmium (II), chromium (III), cobalt (II) and lead (II) ion selective electrodes [19–22] respectively were applied for the determination of trace levels of Cd, Cr, Co and Pb.

The continuous application of these modified working electrodes includes the analysis of steroid and pharmaceutical samples. Fullerene-C_{60} modified electrode as a sensitive voltammetric sensor for the detection of nandrolone [23] and sensitive voltammetric sensor for corticosteroid triamcinolone [24] steroids. The wide applications of electroanalytical techniques also include the analysis of the pharmaceutical assay [25,26]. In situ surfactant modified multiwalled carbon nano-tube paste electrode was applied for acetaminophen, aspirin & venlafaxine, desvenlafaxine by Nafion–carbon nanotube composite glassy carbon electrode and Fullerene-C_{60} modified edge plane pyrolytic graphite electrode for the determination of dexamethasone in pharmaceuticals [27–29]. Gold, silver nanoparticles modified glassy carbon paste electrode sensors were applied for the determination of dopamine, uric acid in urine and bold serum samples [30,31] and polygraphite electrode, fullerene C_{60} coated gold electrode was applied for the determination of 2′, 3′-dideoxyadenosine, dopamine [32,33] in commercially available pharmaceutical formulations respectively.

The electrochemical biosensors, developed during the last decades, offer a new approach to organophosphorus pesticides quantification. Based on the enzymatic inhibition method some of the electrochemical biosensors were developed for the study of organophosphorus pesticides. Cholinesterase-based biosensors were considered as one of the best alternatives in the context of this strategy [34]. Electrochemical sensor based on acetylene black–chitosan composite film modified electrode [35], amperometric silica sol–gel immobilized mono enzymatic acetylcholinesterase biosensor [36], amperometric nano-structured polymer membrane containing gold nanoparticles modified acetylcholinesterase biosensor [37], amperometric screen-printed cross linking biosensor [38], covalent binding [39], direct physical adsorption onto a solid support [40], and encapsulation into a hydrogel [41] biosensors, have been applied for continuous monitoring of pesticide residues in the environmental samples. However the developments of these acetylcholinesterase enzyme biosensors require costlier enzyme and suitable substrate. The lipase enzyme is also inhibited by some of the organophosphorus pesticides and the indirect determination of pesticide concentration by the lipase enzyme is based on effective inhibition of catalytic nature of lipase towards the esteric bonds. Based on this, some of the lipase enzymatic surface acoustic wave impedance sensor [42] and potentiometric biosensor [43] were also developed.

To overcome the above problem, an indirect method of mono-enzymatic liquid state electrochemical biosensor was developed for the determination of Chlorfenvinphos and Malathion pesticides. The developed method is purely based on the production of the *p*-Nitrophenol by *p*-Nitrophenyl Acetate hydrolysis by free lipase enzyme and the pesticide affected lipase enzyme was studied by both cyclic and differential pulse voltammetric techniques.

2. Experimental part

2.1. Reagents and solutions

All chemicals were obtained from commercial sources and used without further purification. Lipases from *Candida rugosa* (EC 3.1.1.3, type VII, ≥700/mg), *p*-Nitrophenyl Acetate, Chlorfenvinphos and Malathion were purchased from Sigma-Aldrich Chemicals. The Chlorfenvinphos and Malathion pesticide stock solutions were prepared by dissolving in acetone (GR grade). The graphite fine powder was procured from Sigma Aldrich and silicon oil, sodium dihydrogen phosphate, disodium hydrogen phosphate and acetone (GR grade) were procured from Himedia chemicals. 0.1 M phosphate buffer was prepared by using 0.1 M disodium hydrogen phosphate and 0.1 M sodium dihydrogen phosphate. All chemicals were of analytical grade and the aqueous solutions were prepared with double distilled water. The enzyme stock solutions were preserved at −5 °C.

2.2. Apparatus

Cyclic voltammetric experiments were performed with a CH-Instruments (USA) model no CHI610D Electrochemical work station with a connection to a personal computer was used for the electrochemical measurement and treating of data. A conventional three electrode cell was employed throughout the experiments, with a bare carbon paste electrode (homemade cavity of 3.0 mm diameter) as a working electrode, saturated calomel electrode (SCE) as a reference electrode and a platinum wire as a counter electrode. All the experiments were carried out at room temperature.

2.3. Preparation of bare carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of 70% fine graphite powder and 30% silicon oil in an agate mortar to produce a homogenous carbon paste. The paste was packed into the cavity of homemade PVC (3 mm in diameter) and then smoothed on a weighing paper. The electrical contact was provided by copper wire connected to the paste at the end of the tube [44].

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