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Sensors and Actuators B 105 (2005) 81–87
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Early-warning electrochemical biosensor system for environmental monitoring based on enzyme inhibition

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Available online 8 April 2004

Abstract

Potentiometric and conductometric biosensors based on immobilised cholinesterases have been developed. Detection of some organophosphorous pesticides (methyl parathion, methyl paraoxon, ethyl paraoxon, trichlorphon, diisopropyl fluorophosphates) and carbamate pesticide (carbofuran) have been realised through enzyme inhibition mechanisms.

Comparison of sensitivity of the biosensor systems developed with that of traditional analytical techniques (high performance liquid chromatography and Lumistox test based on *Vibrio fischeri* luminescent bacteria*)* has shown that biosensor measuring procedures are well suited for fast environmental control. Analytical characteristics of the systems created, their advantages and shortcomings are discussed.

Keywords: Electrochemical enzyme biosensor; Cholinesterases; Organophosphorous and carbamate pesticides; Enzyme inhibition mechanism

1. Introduction

Tons of pesticides annually used in agriculture and horticulture can degrade in the environment by microbial- and photodegradation, and chemical hydrolysis. During these transformation processes, intermediate products, that may be more toxic than the initial products, are generated. These toxic compounds contaminate air, soil and water over large areas. Consequently, it is necessary to develop tests for toxicity assessment of environmental samples.

Currently, a huge array of analytical methods for toxic agent's detection is used. These methods, based on spectrophotometry, chromatography, mass spectrometry and various hyphenated techniques, require sophisticated and expensive equipment, highly trained staff and are usually time-consuming [1,2]. During the last 10–15 years a number of biosensors for determining different pesticides in aqueous solutions have been proposed and developed, in particular, biosensors based on ion-selective and pH-sensitive glass and metal electrodes [3,4], conductometric planar electrodes [5] and pH-sensitive field-effect transistors [6]. However, all these devices are monobiosensors, i.e. only pesticides of a definite type can be determined by each of them, while a mixture of toxic compounds is usually present in real samples. Multibiosensors, sensors consisting of several transducers with different bioselective membranes, can be a solution for the simultaneous determination of various pollutants.

This paper describes a biosensor system based on two types of transducers, potentiometric pH-sensitive field-effect transistors and conductometric thin-film interdigitated electrodes, incorporating two enzymes, acetylcholinesterase and butyrylcholinesterase. This biosensor system was used for the detection of organophosphorous and carbamate pesticides and their photoderadation products. Results are compared with those obtained using standard analytical methods and a bio-test based on luminescence bacteria for toxicity assessment.

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0925-4005/$ – see front matter
doi:10.1016/j.snb.2004.02.039

2. Experimental

2.1. Chemicals and reagents

Acetylcholinesterase (AcChE) (EC 3.1.1.7, type VI-S: from Electric Eel) with a specific activity of 292 U/mg solid; butyrylcholinesterase (BuChE) (EC 3.1.1.8, from Horse Serum) with a specific activity of 13 U/mg solid; bovine serum albumin (BSA) (fraction V, 98% purity), acetyl choline chloride (AcChCl) (99% purity), butyryl choline chloride (BuChCl) (98% purity) and glutaraldehyde (grade II, 25% aqueous solution) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). 2-Pyridinealdoxime methiodide (99% purity) was from Aldrich (Milwaukee, WI, USA).

Organophosphorous pesticides trichlorfon (dimethyl-(2,2,2-trichloro-1-hydroxyethyl) phosphonate), diisopropyl fluorophosphate (DFP), paraoxon-ethyl (*O*,*O*-diethyl *O*-(4-nitrophenyl) phosphate), paraoxon-methyl (*O*,*O*-dimethyl *O*-(4-nitrophenyl) phosphate), parathion-methyl (*O*,*O*-dimethyl *O*-(4-nitrophenyl) thiophosphate) and the carbamate pesticide carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were obtained from Riedel-de Haen GmbH (Seelze, Germany). 4-Nitrophenol (98% purity) was obtained from Acros Organics (Geel, Belgium). All other reagents were of analytical grade and were used without any further treatment.

2.2. Sensor design

The planar conductometric transducers were fabricated at the Institute of Chemo- and Biosensorics (Munster, Germany, Fig. 1a) [7]. Two pairs of Pt (150 nm thick) interdigitated electrodes were made by the lift-off process on the Pyrex glass substrate. The Ti intermediate layer of a 50 nm thick was used to improve adhesion of Pt to substrate. Central part of the sensor chip was passivated by Si_3N_4 layer to define the electrodes working area. Both the digits width and interdigital distance were 10 μm, and their length was about 1 mm. Thus, the sensitive part of each electrode was about 1 mm^2.

The ion sensitive field effect transistors were fabricated at the Research Institute of Microdevices (Kiev, Ukraine). The potentiometric sensor chip contains identical Si_3N_4-ISFETs (Fig. 1b). The design and operation mode have been described previously [8]. The ISFETs were operated at a constant source current and drain–source voltage mode (I_s = 100 μA, V_{ds} = 1 V). The bare substrate of the sensor chip was used as a quasi-reference electrode.

2.3. Enzyme immobilisation

Biologically active membranes were formed by cross-linking enzyme with BSA on the transducer surface in a saturated glutaraldehyde vapour [9,10]. The mixture containing 5.0% (w/v) enzyme, 5.0% (w/v) BSA and 10% (w/v) glycerol in 20 mM phosphate buffer (pH 7.4) was deposited on the sensitive surface of one transducer by a drop method, while a mixture of 10% (w/v) BSA and 10% (w/v) glycerol in 20 mM phosphate buffer (pH 7.4)—on the reference transducer. The sensor chip was then placed in a saturated glutaraldehyde vapour. After 30 min of exposure in glutaraldehyde, the membranes were dried at room temperature for 15 min.

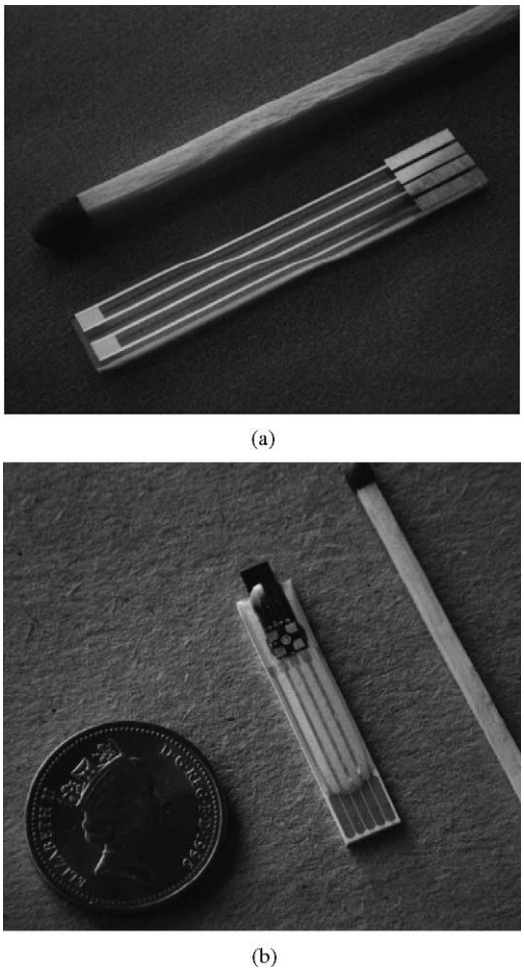

Fig. 1. General view of conductometric (a) and potentiometric (b) transducers.

2.4. Analysis by enzyme biosensor

All measurements were performed in daylight at room temperature in an open vessel filled with the vigorously stirred 5.0 mM phosphate buffer solution, pH 7.4. Concentrations of substrates were adjusted by adding definite volumes of the stock solution of 200 mM AcChCl or 200 mM BuChCl. The solutions of toxic substances were prepared in distilled water and used for enzyme inhibition in another vessel.

The differential output signal between the measuring sensors and the reference sensors was registered with the “home