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Feasibility of application of conductometric biosensor based on acetylcholinesterase for the inhibitory analysis of toxic compounds of different nature

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HIGHLIGHTS

- The conductometric biosensor based on acetylcholinesterase was characterized.
- It was applied for inhibitory analysis of different toxins and toxicants.
- Pesticides, heavy metals, surfactants, aflatoxin and glycoalkaloids were determined.
- Studies showed that the different classes of inhibitors can be distinguished.
- Algorithm of analysis of complex multicomponent samples is proposed.

GRAPHICAL ABSTRACT

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ABSTRACT

This study was aimed at the development of a conductometric biosensor based on acetylcholinesterase considering the feasibility of its application for the inhibitory analysis of various toxicants. In this paper, the optimum conditions for enzyme immobilization on the transducer surface are selected as well as the optimum concentration of substrate for inhibitory analysis. Sensitivity of the developed biosensor to different classes of toxic compounds (organophosphorus pesticides, heavy metal ions, surfactants, aflatoxin, glycoalkaloids) was tested. It is shown that the developed biosensor can be successfully used for the analysis of pesticides and mycotoxins, as well as for determination of total toxicity of the samples. A new method of biosensor analysis of toxic substances of different classes in complex multicomponent aqueous samples is proposed.

1. Introduction

In the recent years, environmental monitoring is becoming an increasingly urgent task. This is due to the rapid development of

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chemical industry and intensive use of various chemicals in agriculture and other fields of human activity. One of the promising trends in research for environmental monitoring is biosensorics, namely the development of enzyme electrochemical biosensors for the determination of different classes of toxicants [1]. A significant interest manifested today to biosensors is due to their specific advantages compared to traditional methods of analysis, i.e., relative cheapness and ease-of-use, high sensitivity and specificity.

To determine the toxic substances by biosensors, an inhibitory analysis based on a variety of enzymes (cholinesterase, urease, glucose oxidase, etc.) is commonly applied. Today acetylcholinesterase (AChE) is the most frequently used enzyme, due to its sensitivity to a number of toxic substances–pesticides, glycoalkaloids, etc. [2].

The first biosensor based on the cholinesterases inhibition was developed in 1962 for determination of agents with neuroparalytic action [3]. Since then, a large number of AChE-based biosensors were developed for determination of various toxic substances. Most of them are designed for inhibitory analysis of organophosphorus pesticides [4], others are reported for identification of heavy metals in model [5,6] and real [7] samples, neurotoxins [8], chemical agents of neuroparalytic action [9,10], drugs [11], etc.

Therefore, a conclusion can be made that AChE is inhibited with very wide range of toxic substances. On one hand, it is an advantage of AChE-based biosensors since it allows their application for analyzing numerous substances. On the other hand, the problems occur for analysis of mixture of toxic substances, i.e., when there is a need to distinguish the impact of various toxicants on AChE. Thus, it is reasonable to analyze the total impact of different toxic substances on the AChE-based biosensor.

This work is devoted to the development and investigation of the AChE-based conductometric biosensor for inhibitory analysis of various toxicants (organophosphorus pesticides, heavy metal ions, surfactants, aflatoxins and glycoalkaloids). The aim of this study was to analyze the different types of inhibition of a bioselective element (reversible and irreversible), to consider the feasibility of application of the AChE-based biosensor for the identification of both particular class of toxicants and complex multicomponent mixtures.

2. Methods of research

2.1. Materials

Bioselective membranes contained enzyme acetylcholinesterase (AChE) from electric eel (EC 3.1.1.7), activity 426 U/mg, bovine serum albumin (BSA) (fraction V, purity ≥ 98.0%), 50% aqueous solution of glutaraldehyde (GA) specially purified for use as an electron microscopy fixative or other sophisticated use (all – from "Sigma–Aldrich Chemie", Germany), and glycerol of domestic production. Acetylcholine chloride (AChCl) (purity ≥ 99%) ("Sigma–Aldrich Chemie", Germany), was used as a substrate. Solutions of trichlorfon (analytical standard) ("Riedel-de Haën", Germany) and aflatoxin B1 (purity ≥ 98%) ("Sigma–Aldrich Chemie", Germany), cationic surfactant benzalkonium chloride (purity ≥ 95.0%) ("Fluka", Sweden), solutions of heavy metal nitrates (domestic production), solutions of crystalline α-solanine and α-chakonin from sprouts of *Solanum tuberosum* ("Sigma–Aldrich Chemie GmbH", Germany) were used as inhibitors.

Solutions of pyridine-2-aldoxime methyl iodide (PAM-2) (purity ≥ 98%) and ethylene diamine tetra acetate (EDTA) (analytical standard) ("Sigma–Aldrich Chemie", Germany) served as reactivators.

Compounds for preparation of buffers and other inorganic substances used in the work were of domestic production and had purity higher than 98%.

2.2. Conductometric transducers

The conductometric transducers (Fig. 1), used in this work, were produced according to the authors' recommendations at the Lashkarev Institute of Semiconductor Physics (Kyiv, Ukraine). They were 5 mm × 30 mm in size and composed of two identical pairs of gold interdigitated electrodes deposited on a ceramic base. The sensitive surface of each electrode pair was approximately 1.0 mm × 1.5 mm. The width of the transducer fingers and the distance between them were 20 mm. The conductometric transducers were connected to the measuring setup, which is described in detail in previous works [12,13].

2.3. Preparation of bioselective elements

A biomembrane was formed by the method of enzyme immobilization developed in our laboratory [14]. For preparing working bioselective membranes, a solution consisting of 1% acetylcholinesterase, 4% BSA and 10% glycerol in 20 mM phosphate buffer, pH 6.5, was used. The mixture for the reference membrane consisted of 5% BSA and 10% glycerol in the same buffer. After deposition of the prepared solutions on working surfaces of conductometric transducers, the latter were placed in saturated glutaraldehyde vapor for 20 min, and then kept for 5 min in air at room temperature. Before using in a biosensor, the membranes were washed with the buffer solution from excess of unbound components.

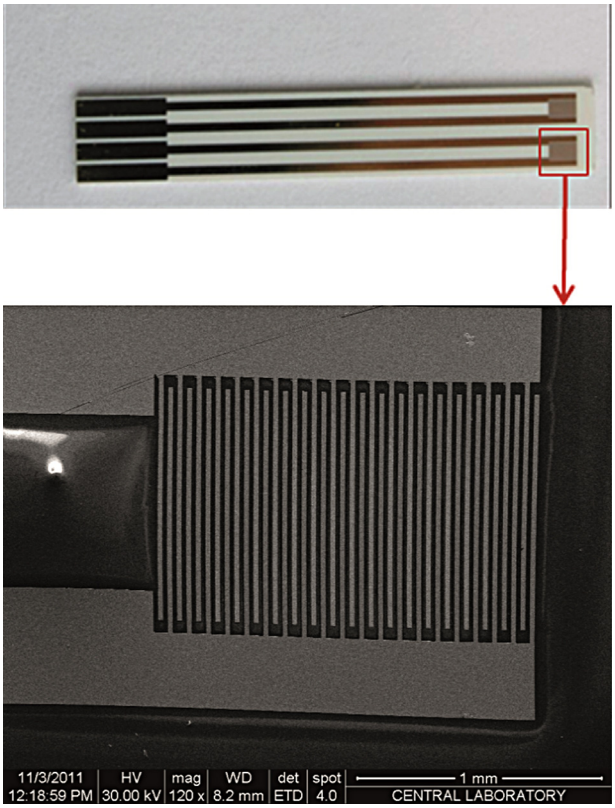

Fig. 1. General view of conductometric transducer and microimages of the gold interdigitated electrodes obtained with a scanning electron microscope.