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Polyaniline-modified cholinesterase sensor for pesticide determination

A.N. Ivanov [a], L.V. Lukachova [b], G.A. Evtugyn [a,*], E.E. Karyakina [b], S.G. Kiseleva [c], H.C. Budnikov [a], A.V. Orlov [c], G.P. Karpacheva [c], A.A. Karyakin [b]

[a] *Kazan State University, Kremlevskaya 18, 420008 Kazan, Russia*
[b] *Moscow State University, 119899, Moscow, Russia*
[c] *A.V. Topchiev Institute of Petrochemical Synthesis, Russian Academy of Science, Leninsky Prospect, 29, 117912, Moscow, Russia*

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Abstract

Cholinesterase sensors based on glassy carbon and planar epoxy graphite electrodes modified with processed polyaniline have been developed and examined for pesticide detection. The modification of electrode surface with polyaniline provides high operational stability and sensitivity towards the pesticides investigated. The detection limits found (coumaphos, 0.002, trichlorfon, 0.04, aldicarb, 0.03, methiocarb, 0.08 mg l^{-1}) make it possible to detect the pollutants in the waters on the level of limited threshold levels without sample preconcentration. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cholinesterase biosensor; Polyaniline; Pesticide determination

1. Introduction

Organophosphorus and carbamic pesticides are widely used in agriculture as insecticides. Certain amount of pesticides, when transferred into the environment, can be dangerous for human health within several weeks [1,2]. Thus, there is a need for fast and inexpensive testing devices for pesticide detection. One of the approaches to their developments assumes the determination of pesticide inhibition on cholinesterases [3–6] (see also review [7]). Previously, we have shown that the use of processed polyaniline doped with camphorsulfonic acid for the modification of carbon electrodes provides reproducible super-Nernstian pH response of about 87 mV per pH unit over the range of 3–9 pH [8]. In this work, the use of polyaniline-modified electrodes in the assembly of cholinesterase sensors has been explored for the detection of enzyme inhibitors.

2. Experimental

Butyrylcholinesterase from horse serum purchased from JSC Biomed (Perm, Russia), 4.2 U mg^{-1}, and from Sigma (St Louis, MO, USA), 500 U mg^{-1}, as well as acetylcholinesterase from electric eel, 1000 U mg^{-1}, from Sigma, were immobilised by cross-linking with glutaraldehyde on the surface of glassy carbon or planar epoxy graphite electrode (IVA, Ekaterinburg, Russia). Prior to enzyme immobilisation, electrodes were covered with 0.5% polyaniline solution in chloroform containing camphorsulfonic acid and phenol [8] and left to dry at room temperature. Acetylcholine iodide was used as the substrate. Trichlorfon was purchased from Sigma, coumaphos, methiocarb and aldicarb from Riedel-de-Haen (Seelze, Germany). All the measurements were performed in 0.002 M Tris buffer solutions containing 0.1 M NaCl.

The potential shift of polyaniline sensor caused by acid release in enzymatic reaction (1),

$$(CH_3)_3N^+CH_2CH_2OC(O)CH_3 + H_2O \rightarrow (CH_3)_3N^+CH_2CH_2OH + CH_3COOH, \quad (1)$$

was measured vs. Ag/AgCl electrode by digital ionometer Ecotest-001 (Econix, Russia) within 5 min after the substrate injection. Before incubation, coumaphos was preoxidized in a 10-min electrolysis to phosphoryl analog as described in [9].

3. Results and discussion

The immobilisation of cholinesterase on polyaniline-modified sensor does not alter the pH sensitivity of the

* Corresponding author. Tel.: +7-8432-387051; fax: +7-8432-380412.
E-mail address: Gennady.Evtugyn@ksu.ru (G.A. Evtugyn).

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