



# Acetylcholinesterase biosensor for inhibitor measurements based on glassy carbon electrode modified with carbon black and pillar[5]arene

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## ABSTRACT

New acetylcholinesterase (AChE) biosensor based on unsubstituted pillar[5]arene (P[5]A) as electron mediator was developed and successfully used for highly sensitive detection of organophosphate and carbamate pesticides. The AChE from electric eel was immobilized by carbodiimide binding on carbon black (CB) placed on glassy carbon electrode. The working potential of 200 mV was obtained in chronamperometric mode with the measurement time of 180 s providing best inter-biosensors precision of the results. The AChE biosensor developed made it possible to detect  $1 \times 10^{-11}$ – $1 \times 10^{-6}$  M of malaoxon,  $1 \times 10^{-8}$ – $7 \times 10^{-6}$  M of methyl-paraoxon,  $1 \times 10^{-10}$ – $2 \times 10^{-6}$  M of carbofuran and  $7 \times 10^{-9}$ – $1 \times 10^{-5}$  M of aldicarb with 10 min incubation. The limits of detection were  $4 \times 10^{-12}$ ,  $5 \times 10^{-9}$ ,  $2 \times 10^{-11}$  and  $6 \times 10^{-10}$  M, respectively. The AChE biosensor was tested in the analysis of pesticide residuals in spiked samples of peanut and beetroot. The protecting effect of P[5]A derivative bearing quaternary ammonia groups on malaoxon inhibition was shown.

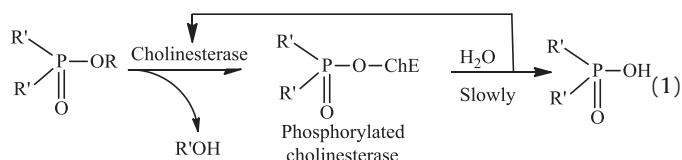
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## 1. Introduction

The detection of pesticides traces in the environment, food and living beings has attracted great attention during recent decades because of high risks of their acute toxicity and possible accumulation followed by long-term damage to humans [1]. Although conventional analytical techniques, especially chromatography and electrophoresis, offer efficient and sensitive detection of extremely low amounts of pesticides in various matrices [2,3], there are certain limitations related to complexity and time-consuming procedures of sample pre-treatment including extraction and use of organic solvents.

Biosensors are integrated receptor-transducer devices providing analytical information using a biological recognition element [4]. Their progress in environmental monitoring and food safety control is mainly caused by demands of field analysis that aimed at fast assessment of potential hazards related to contaminants, natural toxins and synthetic additives [5,6]. The choice of biological element in biosensor assembly depends on biological targeting of toxic species and specificity of detection of their specific interactions. Regarding organophosphate and carbamate insecticides, acetylcholinesterase (AChE) provides best conditions

for quantification due to extremely high sensitivity of the response. Both classes of pesticides form enzyme-inhibitor complex inactive in the reaction with the substrate (see (1) for organophosphate as an example). The rate of spontaneous reactivation of inhibited enzyme is rather slow so that the number of active sites participating in the substrate conversion decreases with the concentration of an analyte.



Changes in the rate of enzymatic reaction correspond to the formal kinetics of irreversible inhibition described by the Alridge equation [7].

$$\ln \frac{\nu_0}{\nu_t} = k_{II} c_I \tau \quad (2)$$

Here,  $\nu_0$  and  $\nu_t$  are the rates of enzymatic reaction prior to and after the contact with the pesticide;  $c_I$  is the pesticide concentration and  $\tau$  the incubation period. The bimolecular inhibition

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