Biosensors for Pesticides detection in Food

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Abstract

The paper is presenting the work related to development of a fully integrated platform for pesticides detection based on amperometric Acetylcholinesteraz (AChE) enzyme biosensors, to be used in food monitoring giving quantitative information on the organophosphate and carbamate pesticides. The sensor design, fabrication, and characterisation and the platform components and functioning will be presented.

Key words: micro-biosensors, integrated platform, pesticide detection, food control.

Introduction

Pesticides used to indicate chemicals, synthetic or natural, that are used for the control of insects, fungi, bacteria, weeds, nematodes, rodent and other pests [1]. The pesticides residues may enter into the food chains through air, water and soil and causes several health problems to ecosystems, including human beings. In the food industry the detection of contaminants, verification of product content, monitoring of raw materials conversion and product freshness are more and more using miniaturised sensors. Enzymatic biosensors based on the selective inhibition of specific enzymes by different classes of toxic compounds are popular in the area of toxins detection. The decrease of activity of the immobilized enzyme in the presence of the analyte is used for its quantification [2].

Sensors fabrication

The miniaturized, planar, amperometric biosensor has been fabricated on silicon substrate using microtechnology techniques and Acetylcholinesterase (AChE) enzyme immobilization on the working electrode. We work on n <100> silicon wafers, and we continue by growing 500nm thermal SiO₂ and successively deposition and patterning of Ti/Pt, Ti/Au and Ti/Ag for three electrodes amperometric transducer fabrication (Fig.1);

Two working electrodes (WE) are patterned on the chip for possible differential measurements or just connecting them together for increasing the active sensitive area. The working electrode used to be functionalized with 3-mercaptopropanoic acid (3-MPA) and 11-mercaptoundecanoic acid (11-MUA), after that following: immersion in EDC/NHS molar report 1:4, 1h for activation of the carboxyl group, cleaning in DI Water, 3 minutes and drying and enzyme deposition using a plotter or immersion for 4h in enzymatic solution.

Testing and results

The biosensors have been tested in presence of tomatoes and grapes with addition of Coumaphos in different concentration. The protocol for biosensor activation and reactions in presence of tomatoes or grapes sample in PBS (1:1) and organophosphate Coumaphos in concentration decreasing from 10⁻⁷mM/L ÷ 10⁻⁴ mM/L is presented in the Table 1. The testing results are presented in Fig.3 and 4.

Additionally, a fully functional automated platform (Fig.5) has been fabricated, able to accommodate different sensors, using a prefilled vessel with 12 mini-chambers, actuated by a step by step motor and to perform Computer control, Data storage, interpretation and alarming.
Conclusions
We obtained a silicon chip biosensor with AChE enzyme immobilised on WE, disposable and allowing the amperometric detection of organophosphate and carbamate pesticides up to the lower limit of 10^{-9} M/l based on inhibition of AChE in vegetables, fruits, and other fresh food products and an automated, mobile, low cost and automated platform to be used on field.

![Fig. 1 - Amperometric transducer layout](image1)

![Fig. 2 – Fabricated device](image2)

![Fig. 3 – Testing results (tomato juice)](image3)

![Fig. 4 – Testing results (grape juice)](image4)

![Fig. 5 – The automated platform](image5)

Table 1 - Biosensor activation and reactions protocol

<table>
<thead>
<tr>
<th>STEP</th>
<th>Description</th>
<th>Reagent</th>
<th>Incubation time (min)</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Equilibration</td>
<td>0.1MPBS, pH=7.2</td>
<td>60 min Recording 20 min</td>
<td>- / -</td>
</tr>
<tr>
<td>2.</td>
<td>Peak generation</td>
<td>ATCh 3mM</td>
<td>10 min</td>
<td>- / -</td>
</tr>
<tr>
<td>3.</td>
<td>Washing</td>
<td>0.1MPBS, pH=7.2</td>
<td>5 min</td>
<td>- / -</td>
</tr>
<tr>
<td>4.</td>
<td>Peak generation</td>
<td>0.1MPBS, pH=7.2</td>
<td>20 min</td>
<td>- / -</td>
</tr>
<tr>
<td>5</td>
<td>Washing</td>
<td>0.1MPBS, pH=7.2</td>
<td>5 min</td>
<td>- / -</td>
</tr>
<tr>
<td>6.</td>
<td>Equilibration</td>
<td>PBS: Tomatoes juice (1:1) or PBS: Grapes juice (1:1)</td>
<td>30 min</td>
<td>- / -</td>
</tr>
<tr>
<td>7.</td>
<td>Peak generation</td>
<td>ATCh 3mM</td>
<td>15 min</td>
<td>- / -</td>
</tr>
<tr>
<td>8</td>
<td>Incubation with Coumaphos</td>
<td>10^{-7} mM/l; 10^{-6} mM/ml 10^{-4} mM/ml</td>
<td>15 min each</td>
<td>- / -</td>
</tr>
<tr>
<td>9.</td>
<td>Regeneration</td>
<td>2-Pyridinealdoxime methiodide 99%</td>
<td>20 min</td>
<td>- / -</td>
</tr>
</tbody>
</table>

References: