Enzyme immobilzed as biosensor in monitoring of the carbamate pesticides

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Carbamates are potent biological agents used extensively in applications ranging from agriculture to medicine and industry. The source of the insecticidal action of the methyl- and dimethylcarbamates is their ability to directly inhibit cholinesterase (ChE) in both insect and mammals. Additionally to their neurotoxicity, the carbamates exhibit biochemical, immunotoxicological and pharmacological effects widely investigated experimental in our research group. A high acute toxicity of these compounds creates a need for fast responding detection system in order to protect human healt during manufacturing and application processes and subsequently sensitive systems for reliable control of food products and environment pollution. Enzyme (ChE) sensors based on the inhibitory property of the carbamates have been found to be simple and inexpensive tools in their monitoring in water and soil. The mode of action of these pesticides is based on irreversible inhibition of acetylcholinesterase and the same principle is utilized for analysis. The activity and stability of cholinesterases, as well as their sensitivity towards a given inhibitor, depend on the type and the source of cholinesterase. Acetyl- or butyryl- cholinesterases have been purified and are now commercially available. An amperometric biosensor for the detection and determination of carbamate pesticides, based on a ferophthalocyanine-modifed carbon electrodes coupled with acetylcholinesterase was used in evaluation of the carbamates levels in area with expected or determined pollution; the results represent the start point in monitoring environmental carbamates and other pesticides using enzyme biosensors.

Introduction

Inhibition of immobilized enzymes in biosensors

Some new problems arise in the use of immobilized enzymes instead of soluble enzymes to detect inhibitors. First of all, the immobilization of an enzyme generally induces conformational modifications that may affect its activity and its sensitivity towards inhibition. Moreover, many reports point out that immobilization induces a decrease in the sensitivity of enzymes towards inhibitors. When using biosensors, the inhibition process is influenced by various new parameters such as microenvironment effects, diffusion limitation and possible interactions between the substrate and/or the inhibitor and the membrane (9, 5).

Unlike biosensors devoted to the determination of substrates, the detection of inhibitors requires the use of low enzyme loading in order to detect very low inhibitor concentrations. Consequently, enzyme electrodes used in such assay must work under kinetic control. This concept is not usual in the design of biosensors for substrate determination where high enzyme loading are used, so that the responses are governed by diffusion constraints.

The mode of action of these pesticides is based on irreversible inhibition of acetylcholinesterase and the same principle is utilized for analysis.

The use of cholinesterase as a sensing element does not allow for the selective detection of a particular pesticide, but rather provides an estimation of the total anthicolinesterase activity of a sample.

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An amperometric biosensor for the detection and determination of carbamate pesticides, based on a ferophthalocyanine-modifed carbon electrodes coupled with acetylcholinesterase was used in evaluation of the carbamates levels in area with expected or determined pollution; the results represent the start point in monitoring environmental carbamates and other pesticides using enzyme biosensors.

$H_3C - COO - (CH_2)_2 N^+ (CH_3)_3 + H_2O \xrightarrow{AChE} HO - (CH_2)N^+ (CH_3)_3 + CH_3 - COOH$

Acetylcholine (AChO) — colina (ChO)

$(CH_3)_3N^+ - (CH_2)_2 - HO + 2\ O_2 + H_2O \xrightarrow{ChO_x} (CH_3)_3N^+ - (CH_2) - C\ H_2O\ OOH + 2\ H_2O_2$

betaine

H_2O_2 catodic reduction 350mV- Ag/AgCl → produce of reduction of H_2O_2

Material and methods

An amperometric biosensor for the detection and determination of carbamate pesticides, based on a ferophthalocyanine-modifed carbon electrodes coupled with AChE (immobilized enzyme biosensors) was used in evaluation of the carbamates levels (2, 3, 8).

Two methods were used: kinetic and incubation method.

The immobilized enzymes biosensor was used to determination of carbofuran concentration (N-methyl carbamate):

The mode of action is based on irreversible inhibition of acetylcholinesterase by carbamates and the same principle is utilized for analysis.

The inhibition process is influenced by various parameters such as time and substrate concentration, their optim values must to be established before the each pesticide determination .

Preliminary results

Optim reaction time for the carbofuran determination is presented in Figure 1.

Positive linear response induced by AChO concentration up to 0.9mM.

Saturation of the enzyme substrate between 1mM – 1,5 mM AChO.

An appreciable enzyme inhibition by substrate begins after 1,6 mM AChO expressed by remission of the intensity.

The pattern suggests the optim substrate concentration between 0,9 mM – 1,6 mM AChO.

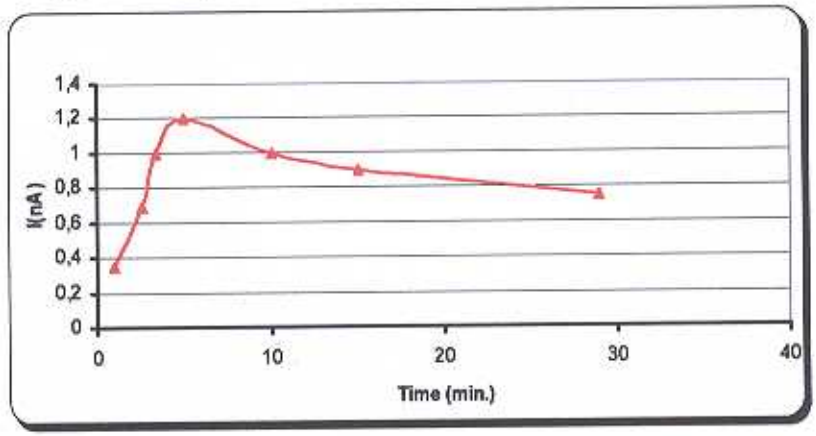

Figure 1

Correlation of the AChE inhibition with the carbofuran concentration **(kinetic method)** is presented in Figure 2.
The pattern indicates:

- A short time / intensity positive dependence with maxim value at 5 min.
- Longer incubation times induced the return to lower values of the reaction.
- The optim reaction time for carbofuran was established 5 min.

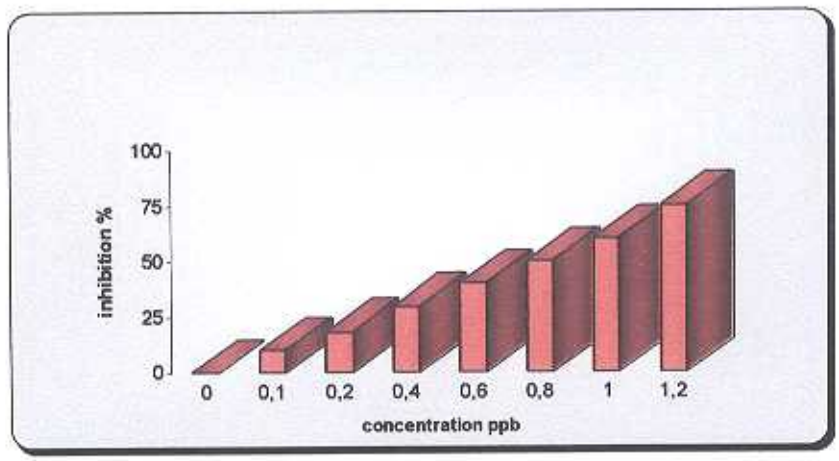

Figure 2

AChE inhibition induced by various carbofuran concentration **(incubation method)** is presented in Figure 3.

- The lowest detectable concentration of carbofuran was limited 0,1 ppb.
- Carbofuran concentration between 0,1 – 1,2 ppb induced a good positive correlation with % AChE inhibition.
- Kinetic method allowed optim work determination of carbofuran concentration was limited between 0,1 – 1,2 ppb

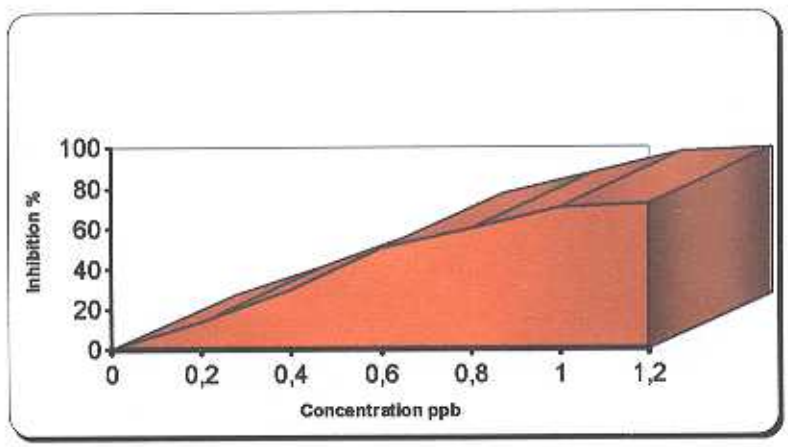

Figure 3

Prelyminary data about influence of the substrat concentration on the intensity of biosensors response is presented in Figure 4.

- Detectable limit concentration of carbofuran beginning about 0,05ppb (lower than those obtained by kinetic method).
- The carbofuran concentration in range 0,1 – 1,2 ppb induced a positive correlation with the AchE inhibition .

- Incubation method allowed optim work determination of carbofuran concentration between 0,05 ppb – 1,2 ppb.

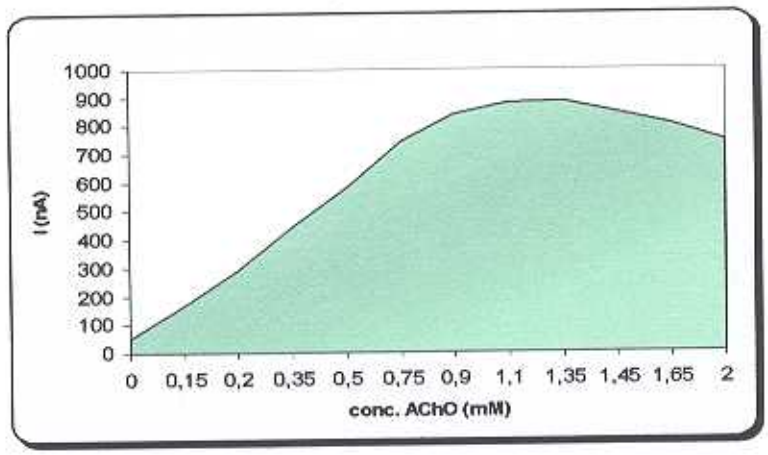

Figure 4

Conclusions

- Enzyme sensors based on the inhibitory property of the pesticides on acetylcholinesterase (AChE) have been found to be simple and inexpensive tools for the detection of pesticides.
- Kinetic method allowed determination of carbofuran in concentration on range limited between 0,1 – 1,2 ppb.
- Incubation method allowed determination of carbofuran concentration in range limited between 0,05 ppb – 1,2 ppb.
- Optim work time for carbofuran determination was established at 5 min;
- The pattern of substrate concentration indicated the optim values between 0,9 mM – 1,6 mM AchO.
- Incubation method - allowing to detect lower concentration (about 0,05 ppb) of carbofuran - was more sensitively compared to kinetic method (0,1 ppb).
- The results represent the start point in monitoring environmental carbamates and other pesticides using enzyme biosensors.

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