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ARTIFICIAL SYNAPSIS – THE DETECTOR OF PESTICIDE TOXICITY

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Abstract – Fast detection of organophosphorous pesticide toxicity in field conditions has not been fully solved yet. The new tool for field measurement of organophosporous pesticides toxicity is described. The detection is based on the inhibition of acetylcholinesterase. The enzyme acetylcholinesterase is immobilized on a miniature electrochemical detector, which is made by Thick Film Technology (TFT). The dimensions of TFT electrochemical sensor are 7,35 x 25,4 mm. The detector is placed in a narrow gap in which the analyzed sample and acetylthiocholine flow. This arrangement simulates the removal of acetylcholin from synaptic gap. It creates the simple model of synapsis - the Artificial Synapsis (AS).

The AS detects the integral sample toxicity. It enables organophosporous pesticides traces to be detected in washout from leaves as well as their direct measurement in rivers, ponds, vaste waters and drinking water sources. The preconcentration of the sample is possible. The detection limit varies in wide range depending on the toxicity of pesticide.

Structure and function of AS is described. Limit of detection (LOD) was found for pesticide Syntostigmin 10^{-10} mol.

Keywords: artificial synapse, microfluidics, organo-phosphorous pesticides

1. INTRODUCTION

Fast detection of pesticide toxicity in field conditions is very important and it is not fully solved yet. The most important aspects are for example the price of one test, the performing place of the test, the time between taking the sample and its analysis, the probability of finding a possitive sample. The Table 1 shows the optimal values of mentioned aspects compared with values in present.

The new tool which can ensure the optimal values of these aspects is presented. It is the AS which is integrated into the biosensor made by TFT technology. The price of one test made by this tool is less then 10 Euro. The device [3], which used this biosensor, is portable. The user can choose the performing place for analysis. The time delay between taking the sample and its analysis is about 30 minutes. The probability of finding a possitive sample is close to 98 %.

TABLE I. Optimal values of aspects compared with present values

Aspect	Present	Optimal
Price of one test	33 Euro	15 Euro
Performing place	Laboratory	Field, laboratory etc.
Time delay of analysis	1 to 5 days	2 hours
Probability of finding a possitive sample	5 %	100 %

The device can ensure an effective prescreening of samples in field or in the laboratory. The full classic analysis will be done only for positive results.

2. THE ARTIFICIAL SYNAPSIS DESCRIPTION

2.1. Toxic activity mechanism

Toxic activity of organophosphorous pesticides is due to Action Potential transfer damage via cholinergic synapsis. The static potential (about – 90 mV) between internal and external side of live cell membrane can be measured there. This potential is the result of ions moving between intracellular (ICT) and extracellular (ECT) liquid. There are cations of Na with higher concentration in ECT, in ICT liquid there are K cations. Both types of cations are transported through the membrane in direction of concentration gradient. The Na pump is repumping cations back against the gradient, because the accumulation of cations should not be there (active transportation). In this case the potential is the same all the time.

If an electrical impulse is brought into the cell, the channels will open and the flow of Na and K cations through these channels rise. In this case the Na pump is not able to repump the increased amount of cations and the membrane will be depolarized, the potential will increase up to + 20 mV. The cell will settle into original steady state after

some time. The potential response arisen by this process is called Action Potential.

Action potentials can arise in muscles and neural cells. They are used for information transport in neural cells. The Action Potential is transmitted among neural cells by a chemical path. The neurotrasmitter is used for this transfer. The transportation with the help of acetycholin (ACH) in cholinergic synapse is a well known example.

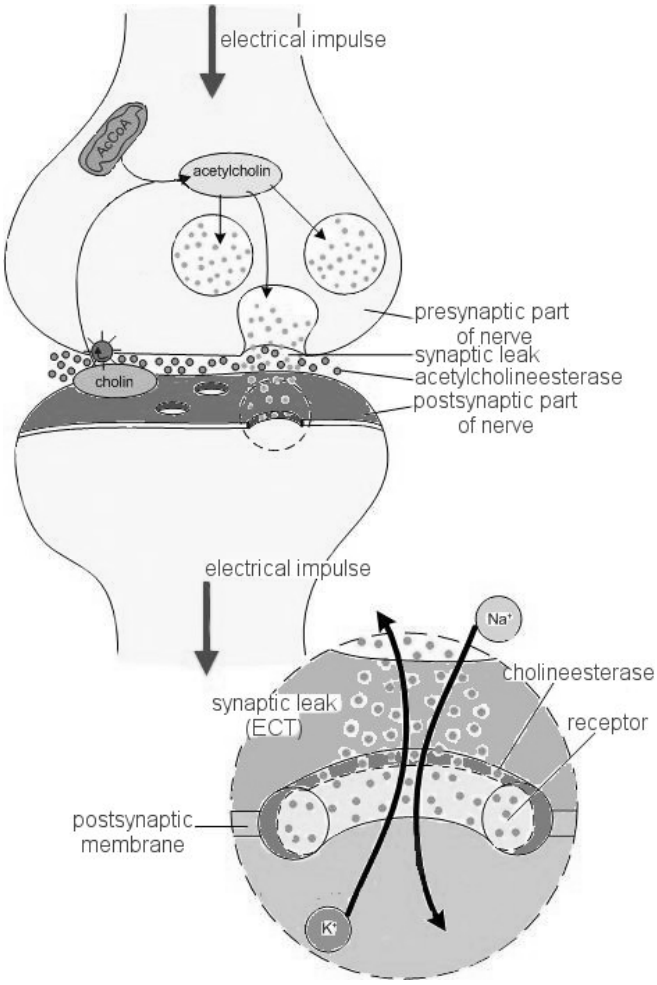

Fig. 1. Neural impulse transmission

Fig. 1 shows neural impulse moving. The ACH is stored in small pockets named vesicules. The total stored amount of ACH is constant, because the synthesis of ACH is constantly changing and compensating the losses made by loosing ACH into the synaptic gap. If the nerve is calm, the content of vesicules is continuously loosing slowly. Since the amount of loose ACH is low, it is immediately disintegrated by the enzyme of acetylcholinesterase (ACHE). The product (choline) is moved by active transportation into presynaptic part of connection. The new ACH is synthesized by enzyme of acetylcholinetransferase and transported into vesicules. If the Action Potential is brought into the end of presynaptic part, the big amount of ACH is loosen. The ACHE is not able to decompose the ACH so fast and the ACH is tied up to receptors of postsynaptic membrane. The Na channel is opened and Na cations flow into the postsynaptic part. The membrane is depolarized and the Action Potential is arisen in the postsynaptic part of the cell, the Action Potencial is transmitted. The ACH is hydrolyzed by cholinesteraze enzyme and the concentration in synaptic gap is reduced. That is why the ACH is loosen from receptors. The ACH hydrolysis must be very fast, because the frequency of neural impulses is hundreds per second.

When the inhibitor (pesticide) penetrates into the nerve, the enzyme function is reduced. The amount of released ACH is continuously increasing in the synaptic gap. The ACH ties up to a receptor. In this case the action potential can be increased without any initial impulse in presynaptic part. And there are more Action Potentials in the output forming the answer to one Action Potential in presynaptic part as shows Fig. 2.

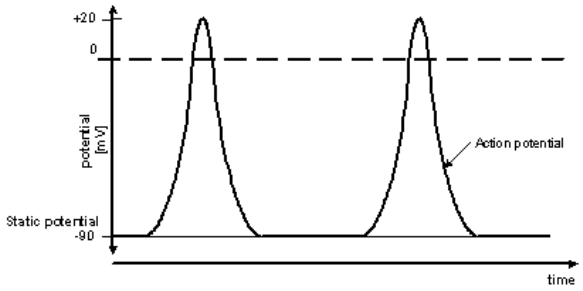

Fig. 2. Action potential seeking for inhibition

The noncoordinated Action Potentials is result, which can invoke cramps or even death. The mentioned function is very simplified and schematic. The aim is to make some intuitive imagination of toxic-activity mechanism. See [1], [2].

2.2 Artificial synapsis

Fig. 3 shows the artificial synapse principle, which is analogical to the biologic synapse in simplified shape. The capillary is used for putting an input solution of acetylthiocholine (ATCH – analogical to ACH) (1), which simulates the presynaptic part.

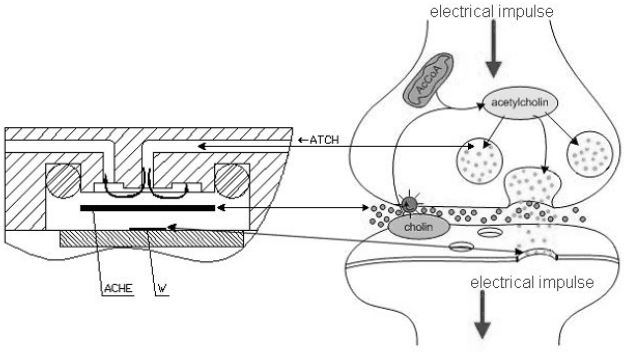

Fig. 3. Artificial synapsis structure

The solution with ACH washes the mechanical membrane with immobilized ACHE the ACH to choline and acetic acid. The product (choline) flows back into the solution (3) and flows to the electrode. It can be detected

there. The choline is not electroactive. When the input substrate is ATCH, which is the substrate with ACHE too, the new product (thiocholine) is electroactive and can be detected by an electrode (2).

2.3 Artificial synapse measurement

The AS is a very effective detector of pesticides. It was integrated into the device [3].

Fig. 4 shows a theoretical course of the measurement on Artificial Synapse. Preparing of solution:

- 10 ml of phosphate buffer (pH = 8),
- 100 µl of Kcl (1 mol),
- 200 µl of ATCH (0,5 mmol).

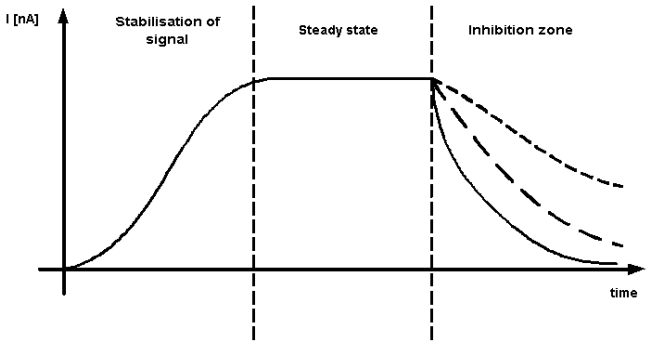

Fig. 4. Theoretical course of the measerement on AS

The solution flows through the cell, where the biosensor is placed. The layer of ACHE enzyme is immobilized on this biosensor and the ACHE starts to react with ATCH. The ATCH decomposes out and the output current goes to steady state in a moment (Stabilisation of signal). When the decomposing of ATCH is constant, the output current is in a steady state (Steady state). This state is the same as static potential in a neural cell. The steady state lasts until the toxic substance (inhibitor) is added into the solution. The ACHE is inhibited by the inhibitor and it is not able to decompose out the ATCH so fast. The current has exponential fall (Inhibition zone). This state is just like autonomic arising of Action Potentials in a neural cell. The frequency of Action Potentials determinates the direction of the current fall. The higher the frequency is, the faster the current falls.

Fig. 5 shows the Artificial Synapse response based on the real measurement.

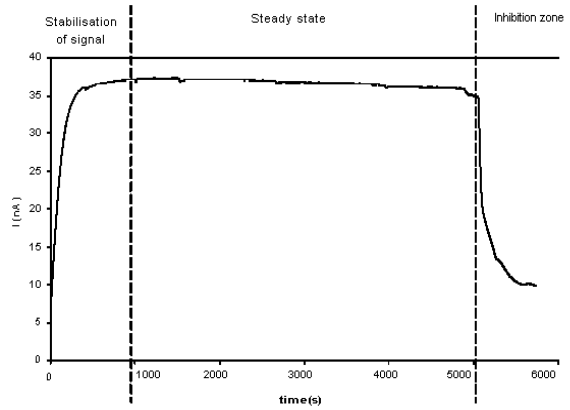

Fig. 5. Real measurement results made by AS

The device is in stabilisation of signal at the time of about 1000 s. The device is in steady state from 1000 s to 5000 s. The current jumps in this zone could have been caused by some bubbles. The inhibitor is added after the current stabilizing at the time of 5000 s and the exponential current fall is shown.

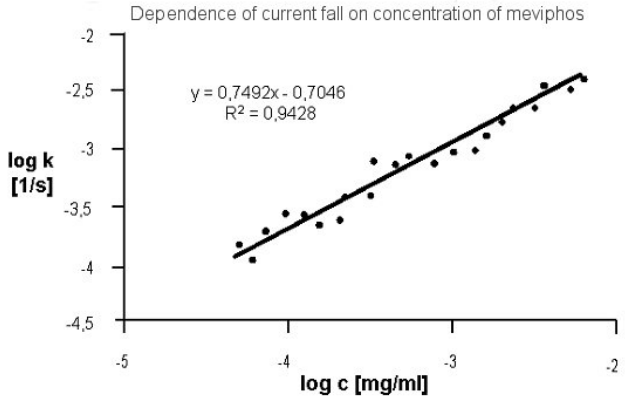

Fig. 6. Calibration curve for mevinphos

Fig. 6 shows the calibration curve for zone (1), which was measured with the laboratory sample of the device. See [4].

3. CONCLUSION

The Artificial Synapsis structure for possibility to determine the pesticide toxicity by biosensor is presented. The strucutre is analogical to biological synapsis. The Artificial Synapsis is integrated in to the biosensor made by Thick Film Technology.

The first testing results made by Articial Synapsis is presented. The main aim is to find calibration values and real measurement course for various types of pesticides. The first results show wide range of detection limit of pesticides toxicity.

The result of the biosensor analysis is the signal corresponding with biologic action of toxic substance. This signal is more valuable for presreening in field conditions then results made by classical laboratory mothods.

The intensive testing measurements are being carried on with various types of pesticides in various concentrations at present time. The detection limit enables to determine the values of pesticide residuum.

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