BIOSENSORS FOR FAST DETECTION OF TOXIC COMPOUNDS

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Abstract

Due to the fact that biosensors play a significant analytical role in agriculture, food safety, environmental and industrial monitoring we present an overview on the biosensor field, especially the design of the interface between the physical transducer and the biological recognition elements for the fast detection of pesticides. We will introduces novel concepts in the area of bioanalysis based on nanomaterials (carbon nanotubes) and composite based on nanomaterials in order to enhance the performance of the developed biosensors. Also, different immobilization procedures for biorecognition element will be presented. Technical description and analytical characterization of the biosensor will be detailed.

Keywords: Sensors, toxic compounds, fast detection

Introduction

Pesticides and phenolic compounds account for 43% of the total amount of organic chemicals used in Europe or worldwide. These compounds are constantly introduced in the environment mainly as a consequence of agricultural and industrial activities and can produce toxic effects towards non-target organisms and flora. Moreover, once they have reached the earth surface, they can suffer biotic and abiotic degradation, with the subsequent formation of transformation products, such are oxo derivatives and sulfoxides, which are more toxic than their parent compounds [1]. Besides, chloro and nitrophenols are the main degradation products of many chlorinated phenoxyacid herbicides and organophosphorous pesticides, respectively. Due to the toxicity and persistence of some of these compounds, the governments and environmental agencies have included different pesticides and phenolic compounds in the environmental monitoring programs by stressing the need of analysing these organic pollutants and transformation products under very low levels of concentration in drinking water and surface water. As a consequence, the achievement of analytical methods, which permit the unequivocal identification, and confirmation of pesticides along with their transformation products in the environment in a quick and reliable way are emerging.

The scientific community concerns in the field of environmental pollutants controlling and monitoring are continuously centring on the research and development of analytical techniques that are able to offer the analytical information, in real time, for a variety of target analytes and matrices. If the 70's, were characterized by the apparition of the first portable gas chromatographs, that permitted the pollutants identification, in the 80's, the analytical chemists developed the immunochemical techniques in clinical laboratories, adapting these techniques to the detection of toxic compounds from environment. In the last decade there was a considerable interest in the development of highly sensitive, selective, rapid and reliable analytical methods, especially for continuous monitoring of the pollutants that present a high level of toxicity. On one hand, current analytical techniques, like gas chromatography and high performance liquid chromatography are very sensitive and reliable. On the other hand,

they are time consuming and expensive. Moreover, only highly trained technicians can perform them.

Biosensors represent an alternative method to detect quickly neurotoxins and they have been an active research area since several years. The unique feature of a biosensor is that the device incorporates a biological sensing element in close proximity or integrated with the signal transducer, to give a sensing system specific for the target analyte [2-3]. The use of this indirect means of assay means that chemically similar solution species can be identified by their biospecific reaction with an immobilized biomolecule such as an enzyme, antibody, nucleic acid, etc. A transducer converts an observed change (physical or chemical) into a measurable signal, usually an electronic signal whose magnitude is proportional to the concentration of a specific chemical or set of chemicals. Those the biosensors combines the specificity of biological systems with the sensitivity of a phisical transducer.

Main Text

Organophosphate and carbamate pesticides that are mainly used in agriculture show low environmental persistence but display high acute toxicity. Their presence in water and food poses a potential hazard to human health. In general, these compounds inhibit acetylcholinesterase (AChE), the enzyme that is responsible for the transmittance of the nervous impulse to the cholinergic synapses [4]. The cholinesterases enzymes are extracted from different sources (*Drosophila melanogaster*, *Electrophorus electricus*, *Caenorhabditis elegans*) and some of them are commercially available.

The working principle of an acetylcholine esterase biosensor is based on the enzymatic hydrolysis of acetyl tiocholine (ATCh) which leads to the formation of acetic acid and tiocholine (TCh) – an electroactive compound. Initial enzyme acitivity is determined by monitoring the formation of TCh. After incubation with pesticide a new measurement is performed after addition of ATCh and the residual enzyme activity is determined.

The detection of the analyte is simply based on the determination of the difference in enzyme activity in the presence and absence of inhibitor, according to the formula reported in figure 1.

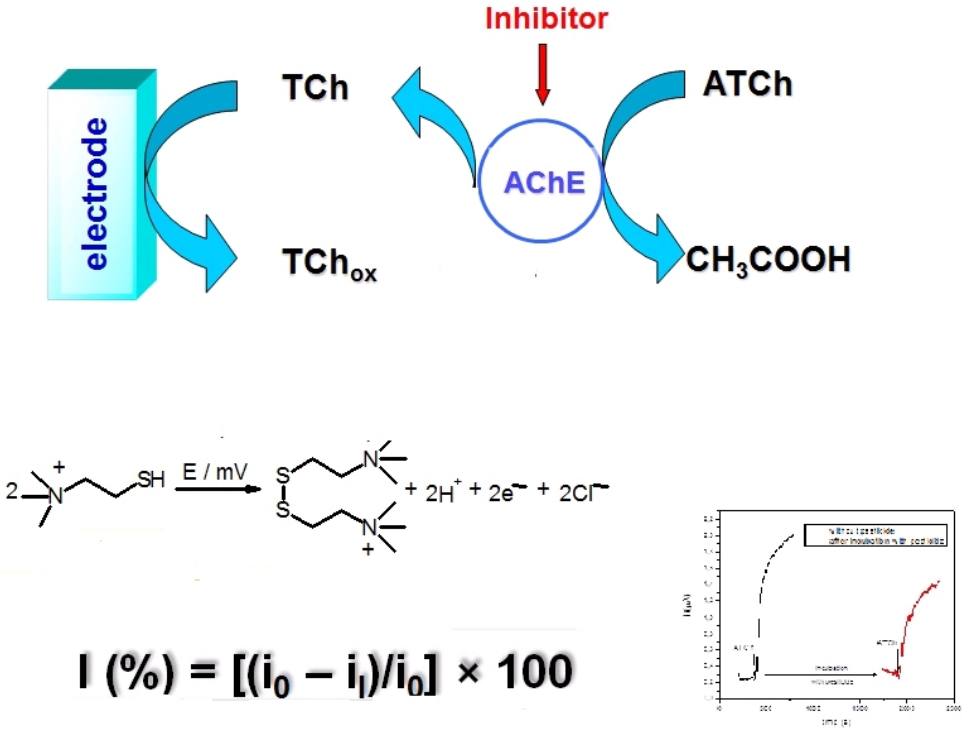

Figure 1. The working principles of the acetylcholinesterase esterase biosensors.

The organophosphoric pesticides inhibit irreversibly the activity of the enzyme acetylcholinesterase, leading to acetylcoline accumulation at post-synaptic membrane level.

The poisoning effect of organophosphate and carbamate pesticides is based on the inhibition of the acetylcholinesterase (AChE) by phosphorylation or carbamoylation of the active site [5]. The enzymatically generated thiocholine (TCh) is an electro-active compound, which can serve as an indicator to reflect the inhibition effect of pesticides. On unmodified carbonbased electrodes, TCh is oxidized at a potential as high as +700 mV vs. Ag/AgCl. The inhibition degree of AChE can be monitored through electrochemically oxidation of enzymatically generated thiocholine (TCh). On unmodified carbon-based electrodes, TCh is oxidized at high potential (+700 mV vs. Ag/AgCl). Such a high value leads to a high background current and can cause interferences from other electroactive species present in analyzed samples.

To develop a biosensor for pesticide we take into consideration several issues. First, the transducer used for detection of TCh, has to provide a fast electron transfer between the analyte and electrode surface in the electrochemical oxidation reaction. Lower applied potential is needed to offer a higher selectivity for TCh detection. Higher sensitivity for pesticide detection could be achieved only if the analytical signal (in our case current) obtained for electrochemical oxidation of TCh is as highest as possible.

Second, the immobilization of AChE should provide an environment that preserves the enzyme activity. In the case of biosensors based on substrate conversion it is recommended a high amount of immobilized enzyme to ensure a high amount of detectable product. On the contrary, in the case of pesticide biosensors the amount of immobilized enzyme should be as low as possible because the inhibition degree, which is proportional with the amount of pesticide, will be higher at a low enzyme loading. TCh concentration should be also very careful settled because the enzyme activity is determined at higher concentration substrate. In this particular case AChE could be inhibited at higher concentration of ATCh. So, ATCH concentration should be optimized in order to avoid inhibition by excess of substrate. And finally, regeneration of biosensor is ofen a requirement.

The electrochemical oxidation of TCh occurs at high applied potential on classical electrodes. Our idea was to try to use a composite gel based on Carbon nanotubes CNT and ionic liquid as electroactive material for a TCh electrochemical sensor.

Carbon nanotubes due to their extraordinary mechanical and electrical properties, high active surface, were extensively used in the last years for sensors and biosensors applications. Ionic liquids (ILs) based on imidazolium cation has been shown to be very promising for electrochemical applications. In these work composite materials based on CNTs, mediators or ILs will be used for electrochemical transducer modification [6-9]. Synergistic effect of the counterparts is exploited in different type of composites in order to improve the analytical performances of composite modified electrodes. The gel between carbon nanotubes and imidazolium ionic liquids act as better materials for improving the electrochemical transfer in electrochemical biosensors.

The immobilization of the active biological component on the transducer surface represents a critical stage in the biosensors development and it has as a goal the settlement of the enzyme on the surface of the physical transducer. The chosen immobilization procedure has to keep the enzyme in the native conformation, especially at active center level, simultaneously ensuring the free diffusion of the substrate, of the reaction product and/or of the inhibitors through the sensitive layer. Due to the fact that during the immobilization step some of the properties of the enzymes may be affected, it is necessary to control rigorously the process. Often, in the case of enzymes immobilization, it was observed a diminishing in enzyme activity due to pH influence, ionic strength or due to the possible conformational modifications that can appear during the process. The immobilization method used for the achievement of an enzymatic biosensor has to fulfill some requirements: to ensure the free

diffusion of the substrate, reaction products and/or inhibitors through the bio sensitive layer; to preserve the enzyme conformation and activity during immobilization process; to ensure a short response time; to preserve the enzyme affinity for substrate and inhibitors.

The most widely used approaches for the immobilization are the adsorption on the surface of the physical transductor, the covalent attachment on a functionalized surface of the sensor, the use of avidin- (or streptavidin-) biotin interactions, or the entrapment in the matrix of a polymer. The immobilization of the enzymes in sol-gel matrix was chosed (figure 2).

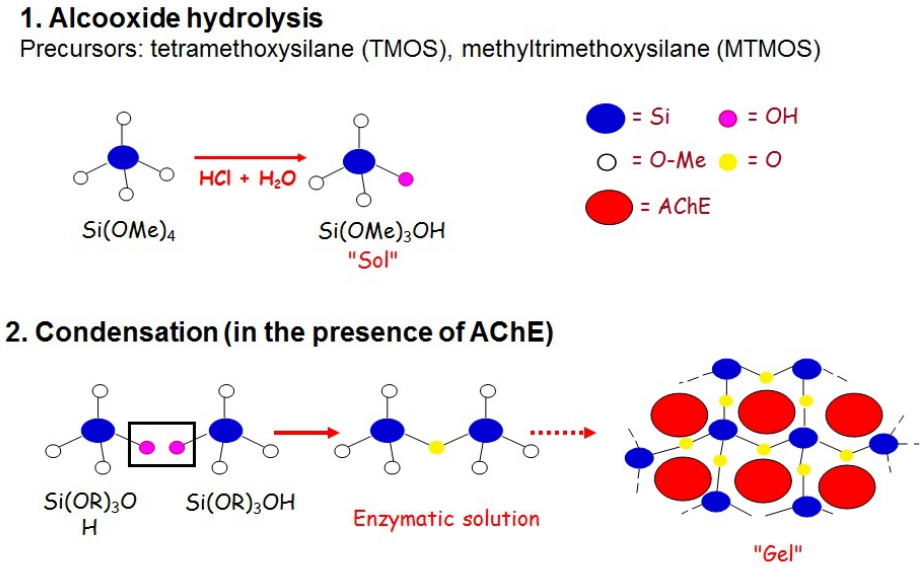

Figure 2. Enzyme immobilization in sol-gel matrix.

Another issue to be address is the regeneration of the enzyme activity in order to reuse the biosensor. The advantages of the regeneration with different oxime compounds will be discussed, especially the regeneration with pralidoxime and obidoxime (figure 3)

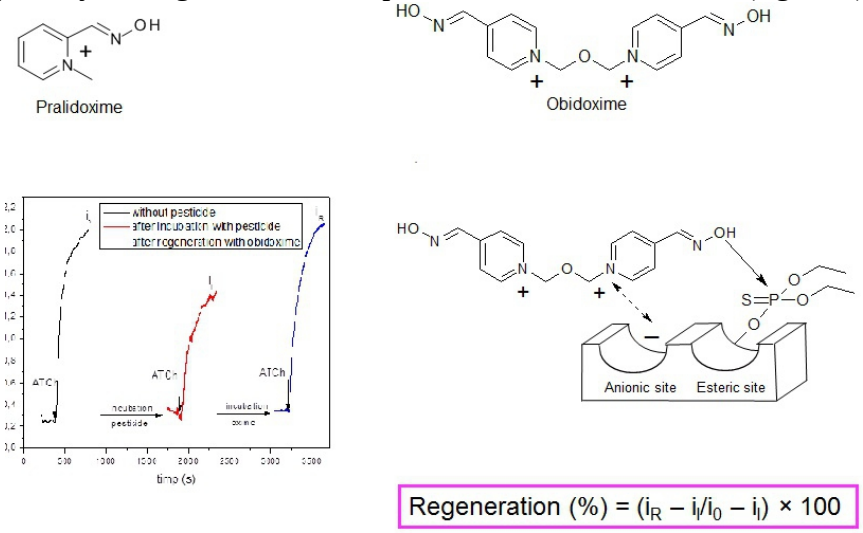

Figure 3. Regeneration principle.

Conclusion

The presentation will highlighted the analytical parameters that should be investigated in order to increase the assay sensitivity using inhibition biosensors. Several applications for the detection of pesticides will be presented.

References:

C. Fest, K.-J. Schmidt, The chemistry of organophosphorus pesticides, reactivity, synthesis, mode of action, toxicology, Springer-Verlag Heidelberg, New York, 1973.

D.R. Thevenot, K. Toth, R. Durst and G.S. Wilson, Electrochemical biosensors: recommended definitions and classifications. Biosensors and Bioelectronics, 16, 121-131, 2001

A. Avramescu, S. Andreescu, T. Noguer, C. Bala, D. Andreescu, J-L. Marty, Biosensors designed for environmental and food quality control based on screen-printed graphite electrodes and different configurations, Analytical and Bioanalytical Chemistry, 374, 25-32, 2002.

A. Amine, L. El Harrad, F. Arduini, D. Moscone, G. Palleschi, Analytical aspects of enzyme reversible inhibition, Talanta, 118, 368-374, 2014.

C. S. Pundir, N. Chauhan, Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review, Analytical Biochemistry, 429, 19–31, 2012.

L. Rotariu, L.G. Zamfir, C. Bala, Low potential thiocholine oxidation at carbon nanotube-ionic liquid gel sensor, Sensors & Actuators: B. Chemical,150, 73–79, 2010.

L. Rotariu, L. G. Zamfir, C. Bala, A novel, sensitive, reusable and low potential acetylcholinesterase biosensor for chlorpyrifos based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotubes gel, Biosensors and Bioelectronics, 26, 3692–3695, 2011.

L. Rotariu, L.G. Zamfir, C. Bala, A rational design of the multiwalled carbon nanotube–7,7,8,8-tetracyanoquinodimethan sensor for sensitive detection of acetylcholinesterase inhibitors, Analytica Chimica Acta, 748, 81-88, 2012.

L.-G. Zamfir, L. Rotariu, C. Bala, Acetylcholinesterase biosensor for carbamate drugs based on tetrathiafulvalene–tetracyanoquinodimethane/ionic liquid conductive gels, Biosensors and Bioelectronics, 46(15), 61-67,2013.