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Chapter 4

Recent Trends in the Development of Electrochemical Biosensors for Organophosphorus Pesticides Determination

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http://dx.doi.org/10.5772/58310

1. Introduction

The environmental and public concerns provoked by the indiscriminate use of organophosphorus pesticides (OPs) and the adopted safety standards [1-6] incited the development of new sensitive methods enabling their determination in the nanomole-picomole range. Such analytical performances offer the nanostructured electrochemical biosensors.

The nanotechnological approach to electrochemical biosensing [7-16], due to the electrocatalytical properties of the nanostructures, their action as electron transfer mediators or electrical wires, large surface to volume ratio, structural robustness, and biocompatibility leads to electrode potential lowering, enhancement of the electron transfer rate with no electrode surface fouling, sensitivity increase, stability improvement, and interface functionalization.

In this review are presented the recent trends in the development of nanomaterials based electrochemical biosensors for organophosphorus pesticides determination. Their performance characteristics such as sensitivity, linear range, detection limits, and stability are compared and discussed.

2. OPs determination applying electrochemical biosensors

The electrochemical biosensors, because of the high sensitivity of the determinations, the simplicity of the operational procedure, the availability and the affordable cost of the equipment, are considered as an alternative to the expensive, time-consuming, and sophisticated chromatographic techniques currently applied for OPs quantification [17].

The main processes involved in the electrochemical biosensors for OPs quantification are: cholinesterases activity inhibition by OPs or OPs hydrolysis catalyzed by organophosphorus hydrolase, both followed by the conversion of the signal produced by the interaction between the biorecognition element and the analyte into electrical one.

2.1. Inhibition based electrochemical biosensors for OPs quantification

The electrochemical biosensors which take advantage of the inhibitory effect of the OPs on cholinesterases activity have been extensively investigated [18-26]. The first generation of inhibition based electrochemical biosensors involves the following reactions:

$$\text{R- choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + \text{R- COOH} \quad (1)$$

$$\text{choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{betaine} + 2H_2O_2 \quad (2)$$

$$2H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (3)$$

or

$$O_2 + 4e^- + 2H_2O \rightarrow 4OH^- \quad (4)$$

where ChE is acylcholinesterase and ChO is choline oxidase.

The acylcholinesterases catalyzed R-choline hydrolysis (Eq. 1) affected by the enzyme activity inhibition with OPs, is coupled with the choline oxidase catalyzed betaine oxidation (Eq. 2). The current of the oxidation of the produced H_2O_2 (Eq. 3) or the current of the reduction of the consumed O_2 (Eq. 4) is registered as a sensor response and is correlated to the OPs concentration.

Nevertheless, the electrochemical biosensors for OPs determination of first generation present some intrinsic deficiencies: sophisticated design as two enzymes have to be integrated, multistep protocol, and possible interferences at the potential of H_2O_2 oxidation (+0.6 V/SCE), among other. These drawbacks imposed the development of the electrochemical biosensors for OPs determination of second generation. They quantify the cholinesterases inhibition applying a simpler measurement principle, consisting in the monitoring of the electroactive thiocholine formed upon enzymatic hydrolysis of acylthiocholine (Eq. 5):

$$\text{R- thiocholine} + H_2O \xrightarrow{\text{ChE}} \text{thiocholine} + \text{R-COOH} \quad (5)$$

The response generating reaction is the direct thiocholine oxidation (Eq. 6) at a potential of+0.8 V/SCE at conventional metal and graphite transducers [27-31]:

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$$2\,\text{thiocholine} \rightarrow \text{dithio- bis- choline} + 2H^+ + 2e^- \quad (6)$$

or the mediated thiocholine oxidation (Eqs. 7 and 8) at lower potentials using chemically modified electrodes [32-40], thus avoiding the interferences:

$$2\,\text{thiocholine} + M_{ox} \rightarrow \text{dithio- bis- choline} + M_{red} \quad (7)$$

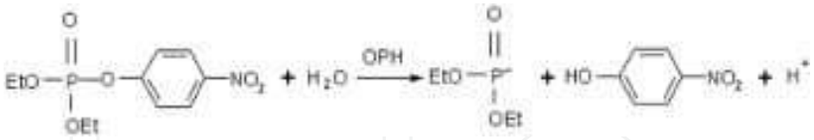
$$M_{red} \rightarrow M_{ox} + 2e^- \quad (8)$$

where M_{ox} and M_{red} are the oxidized and the reduced forms of the mediator M.

The biosensors based on the inhibitory effects of OPs on cholinesterases activity are very sensitive, but the indirect sensing mechanism they use is associated with some shortcomings such as poor selectivity, an irreversible response, etc. The non-ideal behavior of the enzyme inhibition-based biosensors and biosensing systems for OPs determination is exhaustively commented by Luque de Castro [41].

2.2. Substrate based electrochemical biosensors for OPs quantification

The nitro phenyl-substituted OPs (paraoxon, parathion, methyl parathion, fenitrothion, etc.), and some chemical warfare agents (sarin, soman, tabun, VX, etc.) act as substrates for the enzyme organophosphorus hydrolase (OPH) [42-44]. The enzyme catalyzed hydrolysis of these substances yields p-nitrophenol (PNP). The PNP oxidation current, which is the sensor signal, measured at a fixed-potential is proportional to the OPs concentration. The occurring reactions, selecting paraoxon as a model, are the following:



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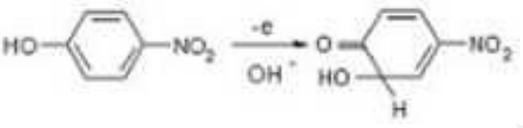

(10)

Thus, the use of OPH is extremely attractive for the direct and selective biosensing of OPs [19, 21, 22, 45-48]. Nevertheless, the sensitivity of the OPH based electrochemical sensors is lower and their detection limits are much higher than those of the inhibition based ones [20, 49]. The PNP oxidation that produces phenoxy radicals which couple to form an insulating polymeric film fouling the electrodes surfaces and inhibiting further phenols oxidation [50-59] and the complex, long-lasting, and expensive procedure for OPH extraction and purification, performed in specialized microbiological laboratories (this enzyme is not commercially available) [21] create additional problems.

3. Nanostructured electrochemical biosensors for OPs quantification

The bibliographical survey covering the period 2010-2013 demonstrated that the predominant part of the recently developed nanostructured electrochemical biosensors for OPs quantification make use of carbon nanotubes (CNTs) or gold nanoparticles (GNPs). Previous studies are revised and reported in the comprehensive reviews of Liu et al. [60] and Periasami et al. [61].

3.1. Carbon nanotubes

Carbon nanotubes are widely used in electrochemical biosensors because of their chemical stability, mechanical strength and stiffness [62, 63], and improved electron transfer properties attributed to the changes in the energy bands close to the Fermi level [64].

The single-walled carbon nanotubes (SWCNTs) display higher surface area and low electrical percolation thresholds in comparison to the multi-walled carbon nanotubes (MWCNTs). Nevertheless, their higher cost and poorer dispersibility limit their application. Moreover, SWCNTs form less regular layers onto the electrodes with a higher deviation of the signal measured. Nevertheless, data reported in the literature [65] demonstrate that OPH covalently immobilized on SWNTs conserves much higher activity than OPH conjugated to MWNTs. This was attributed to the more uniform deposition of OPH on the SWNTs and the formation of a SWNTs network. The dynamic concentration range for paraoxon determination applying SWNT-OPH sensor was found to be in the range 0.5-8.5 μmol L^{-1} with a detection limit of 0.01μg mL^{-1}. In addition, the SWNTs sensor with covalently immobilized enzyme exhibited enhanced solution-storage and operational stability: 25% signal loss over 7 months.

Earlier studies has also shown that the CNT surface modification could be useful for improving the sensitivity and stability of oxidative measurement of phenolic compounds, produced upon OPH catalyzed hydrolysis of OPs. The evaluation of the performances of the SWCNTs and of MWCNTs prepared by chemical vapor deposition (SWCNT-CVD and MWNT-CVD), and by the ARC discharge method (MWNT-ARC) demonstrates that both the SW-and MW-CVD-CNT coated surfaces offer sensitivity enhancement compared to the ARC-CNT and bare electrodes [66]. It was considered that the higher sensitivity of the CVD-CNT-modified electrode reflects differences in the density of edge-plane-like defects that leads to higher electrochemical reactivity [66].

SWCNTs were also used for the development of an inhibition based biosensor for OPs determination, applying a simple protocol. It includes the one-stage deposition of SWCNs and Co phtalocyanine followed by carbodiimide binding of acetylcholinesterase, providing directed coordination of the protein molecule at the terminal carboxylic groups of the oxidized SWCNTs [67]. The biosensor made it possible the detection of 5-50 ppb of paraoxon and 2-50 ppb of malaoxon with detection limits of 3 and 2 ppb, respectively. The amperometric measurements were performed at low potential (0.050 V/Ag, AgCl), thus avoiding the interferences.

The use of pristine MWCNTs and of MWCNTs modified with metal nanoparticles has attracted much attention. Au, Pt, Pd, and Ni nanoparticles are applied to enhance the performances of the CNTs modified electrodes, because of their high catalytic activity, biocompatibility, and

http://dx.doi.org/10.5772/58310

increased surface area. Increase of enzyme loading, promotion of electron transfer, and synergistic effect in the biosensing of methyl parathion were observed using a nanocomposite sensing film prepared via the formation of gold nanoparticles on silica particles, mixing with multiwall carbon nanotubes and subsequent covalent immobilization of methyl parathion hydrolase. A linear response was obtained in the range from 0.001 μg mL^{-1} to 5.0 μg mL^{-1} with a detection limit of 0.3 ng mL^{-1} [68].

The one-step fabrication of MWCNTs-GNPs composite could be performed by in situ reduction of $HAuCl_4$ by $NaBH_4$. The self-coating of the GNPs on the MWCNTs produced a uniform nanocomposite. It was used for the fabrication of an acetylcholinesterase based electrochemical sensor for malathion determination [69]. The detection limit was found to be 0.6 ng mL^{-1}.

3.2. Gold nanoparticles

GNPs are extensively used in biosensors application, for the reason of their biocompatibility, catalytic activity, excellent conductivity, and high surface area [70, 71].

Various materials modified with GNPs were tested as enzyme immobilization matrices. Marinov et al. [72] suggest the use of GNPs loaded chemically modified poly(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membranes (PAN) as supports for acetylcholinesterase immobilization. Since PAN is not electroconductive, GNPs acted as electron transfer "wires". The high enzyme loading and the presence of GNPs resulted in high sensitivity. The detection limit for paraoxon determination was found to be 0.074 ng L^{-1} and a linear response was obtained in the range 0.1-100 ng L^{-1}. Important advantage of the developed biosensor is the substitutability of the enzyme membrane, as the enzyme carrier is a separate element that could be incubated in a pesticide solution and reactivated in PAM solution afterwards aside from the working electrode, which is hence available to be assembled with another enzyme membrane and used for further pesticide measurements.

Simple and efficient strategy for acetylcholinesterase immobilization onto a composite film of Au-polypyrrole nanowires was proposed by Gong et al. [73]. It is assumed that the three-dimensional interlaced polypyrrole nanowires network provides a favorable microenvironment to maintain the bioactivity of the enzyme, while the GNPs distributed in the network-structured matrix facilitate the electron transfer. The inhibition of methyl parathion was found to be proportional to its concentration ranging from 0.005 to 0.12 and 0.5 to 4.5μg mL^{-1} with a detection limit of 2 ng mL^{-1}. After 30-days storage the sensor retained 60% of its initial current response.

A fast method for the preparation of acetylcholinesterase-GNPs-$CaCO_3$ bioconjugates was suggested by Chauhan et al. [74]. The procedure includes the preparation of the hybrid GNPs-$CaCO_3$ material by $CaCO_3$ dispersion into Au colloid solution, followed by enzyme adsorption and encapsulation of the bioconjugates on the electrode surface using silica sol. The electrochemical measurements were performed at a potential of+0.2 V/Ag, AgCl, this avoiding the interferences. It was demonstrated that malathion and chlorpyrifos could be detected in the range of 0.1-100 nM and 0.1-70 nM, respectively, with a detection limit of 0.1 nM for both. The response current of the sensor decreased to 60% after 60 days.

A stable and sensitive inhibition based sensor for OPs quantification was fabricated by Sun et al. [75], using hollow gold nanospheres (HGNs). The protocol comprised glassy carbon electrode modification with chitosan, hollow gold nanospheres adsorption onto the surface of chitosan through electrostatic interactions, L-cysteine assemblage on HGNs through Au-S bond, and acetylcholinesterase covalent immobilization via the-COOH groups of L-cysteine. The inhibition rates of pesticides were found to be proportional to their concentrations in the range of 0.1-150 and 0.1-200 μg L^{-1} for chlorpyrifos and carbofuran, with detection limits of 0.06μg L^{-1} and 0.08 μg L^{-1}, respectively. After 40-days of storage, the sensor retained 85.4 % of its initial current response.

3.3. Other nanomaterials

Effective devices for OPs determination were developed using functionalized graphene structures. It has been demonstrated that the acetylcholinesterase sensors based on graphene oxide, GNP-graphene oxide, and nanoparticles (NiO, Pt, SnO_2)-graphene nanocomposites show high electron mobility, catalytic activity, and sensitivity [76-80]. They were successfully applied for methylparathion, chlorpyrifos, malathion, and dichlorvos quantification. Another sensitive acetylcholinesterase sensor was fabricated using oxidized exfoliated graphite nanoplatelet (xGnPs)-chitosan cross-linked composite [81]. It was used for chloropyrifos determination with a detection limit of $1.58x10^{-10}$ M.

Other carbonaceous materials used in OPs biosensing are the mesoporous carbons and carbon black [82]. The well-ordered nanopores, many edge-plane-like defective sites, and high surface area of the mesoporous carbon resulted in increased sensitivity, and allowed for nanomolar-range detection of the analyte paraoxon using an OPH-based sensor. The detection limit achieved was of 0.12μM (36 ppb).

The potential of the magnetic nanoparticles was exploited for the construction of a disposable acetylcholinesterase-coated Fe_3O_4/Au magnetic nanoparticles (GMP-AChE) sensor [83]. The GMP-AChE were absorbed on the surface of a screen printed carbon electrode modified by carbon nanotubes (CNTs)/nano-ZrO_2/prussian blue(PB)/Nafion (Nf) composite membrane by an external magnetic field. The biosensor exhibited a fast response, wide linear detection range and high sensitivity to OPs due to the conductive Fe_3O_4/Au NPs providing a large electrode surface. The other advantages of the biosensor are associated with the specific adsorption of OPs by the ZrO_2 NPs, and the easy removal and replacement of the Fe_3O_4/Au/AChE by applying an external magnetic field. The biosensor was used for dimethoate determination in the range $1.0x10^{-3}$-10 ng mL^{-1} with a detection limit of $5.6x10^{-4}$ ng mL^{-1}.

An OPs biosensor fabricated by covalent acetylcholinesterase immobilization onto iron oxide nanoparticles and carboxylated multi walled carbon nanotubes modified Au electrode was reported by Chauhan et al. [84]. The synergistic action of Fe_3O_4 NP and MWCNT showed excellent electrocatalytic activity at low potential (+0.4 V). Under optimum conditions, the inhibition rates of OPs were proportional to their concentrations in the range of 0.1-40 nM, 0.1-50 nM, 1-50 nM and 10-100 nM for malathion, chlorpyrifos, monocrotophos and endosulfan, respectively. The detection limits were 0.1 nM for malathion and chlorpyrifos, 1 nM for monocrotophos, and 10 nM for endosulfan. The biosensor was stable (2 months) and reusable (more than 50 times).

http://dx.doi.org/10.5772/58310

4. Analytical performances of the recently developed nanostructured electrochemical biosensors for OPs quantification

The analytical performances of the recently developed nanostructures electrochemical biosensors for OPs quantification are summarized in Table 1.

Electrode/matrix	Technique	Immobilization method	LOD, µM	Linearity, µM	Analyte	Storage stability	Ref.
SWCNT/Co phtalocyanine	amperometric	covalent	0.010 $9.5x10^{-3}$	0.018-0.181 $9.5x10^{-3}$-0.16	paraoxon malaoxon	3 months	67
MWCNTs–Au nanocomposites/GCE	amperometric	hydrophilic surface for biomolecule adhesion	$1.81x10^{-3}$	$3.0x10^{-3}$-3.027	malathion	30 days	69
AuNPs-PAN/Pt	amperometric	glutaraldehyde	$2.69x10^{-7}$	$(3.63\text{-}363)x10^{-7}$	Paraoxon	50 days	72
AuNPs-PPy/GCE	voltammetric	adsorption	$6.86x10^{-3}$	$(17.18\text{-}41.2)x10^{-3}$ 1.71-15.4	methyl parathion	30 days	73
AuNPs-$CaCO_3$/Au	amperometric	adsorption	$0.1x10^{-3}$ $0.1x10^{-3}$	$(0.1\text{-}100)x10^{-3}$ $(0.1\text{-}70)x10^{-3}$	malathion chlorpyrifos	60 days	74
HGNs/CHIT/GCE	voltammetric	covalent	$1.71x10^{-4}$	$2.85x10^{-4}$-0.43	Chlorpyrifos	60 days	75
NiO NPs-Carboxylic graphene-nafion/GCE	amperometric	entrapment	$5x10^{-8}$	$1.0x10^{-7}$-$1x10^{-4}$ $1x10^{-10}$-$1x10^{-8}$	methyl parathion chlorpyrifos	30 days	76
NiO NPs-Carboxylic graphene-nafion/GCE	amperometric	entrapment	$5x10^{-8}$	1×10^{-13}-1×10^{-10} 1×10^{-4}-1×10^{-2}	methyl parathion	30 days	77
SnO_2 NPs-Carboxylic graphene-nafion/GCE	amperometric	entrapment	5×10^{-8}	$1x10^{-7}$-$1x10^{-4}$ $1x10^{-4}$-$1x10^{-2}$	methyl parathion	30 days	78
Graphen oxide-AuNPs/GCE	amperometric	covalent	$2.85x10^{-4}$	$(1.42\text{-}28.5)x10^{-3}$ 0.028-0.285	chlorpyrifos	30 days	79
Graphen oxide-nafion/GCE	amperometric	adsorption	$9x10^{-3}$	$(22.6\text{-}452)x10^{-3}$ 4.52-90.5	dichlorvos	30 days	80
Graphite nanoplatelet–CHIT composite/GCE	voltammetric	covalent	$1.58x10^{-4}$	$1x10^{-4}$-1.0	chloropyrifos	10 days	81
AuNPs/PB/ZrO_2/CNTs/SPCE	DPV	adsorption	$2.44x10^{-6}$	$4.4x10^{-6}$-$4.4x10^{-2}$	dimethoate		83
Fe_3O_4NP/MWCNTs/Au	amperometric	covalent	$0.1x10^{-3}$	$(0.1\text{-}40)x10^{-3}$ $(0.1\text{–}50)x10^{-3}$ $(1\text{-}50)x10^{-3}$	malathion chlorpyrifos monocrotophos	60 days	84

Electrode/matrix	Technique	Immobilization method	LOD, μM	Linearity, μM	Analyte	Storage stability	Ref.
AuNPs–$CaCO_3$ bioconjugate/Au	amperometric	adsorption	$0.1x10^{-3}$	$(0.1\text{-}100)x10^{-3}$ $(0.1\text{-}70)x10^{-3}$	malathion chlorpyrifos	90 days	85
Fe_3O_4NP/MWCNTs/ITO	amperometric	covalent	$0.1x10^{-3}$	$(10\text{-}100)x10^{-3}$ $(0.1\text{-}70)x10^{-3}$ $(0.1\text{-}50)x10^{-3}$ $(0.1\text{-}70)x10^{-3}$	malathion chlorpyrifos monocrotophos	90 days	86
AuNPs/PB/GCE	amperometric	adsorption	$3.5x10^{-9}$	$(0.448\text{-}4.48)x10^{-2}$	monocrotophos	30 days	87
AuNPs/GCE	amperometric	adsorption	$7x10^{-3}$	$(28\text{-}170)x10^{-3}$	methamidophos	7 days	88
AuNPs–MWCNTs/GCE		adsorption	$1x10^{-3}$	$(0.1\text{-}7.0)x10^{-3}$			89
PB-CHIT/GCE	amperometric	glutaraldehyde	$0.113x10^{-4}$, $0.703x10^{-4}$ $0.194x10^{-4}$ $0.33x10^{-4}$	$0.45x10^{-4}$-0.045 $0.234x10^{-3}$-0.046 $0.116x10^{-3}$-0.0194 $0.167x10^{-3}$-0.0335	dichlorvos methoate trichlorfon phoxim		90
MWCNTs/AuNPs–CHIT/GCE	Fourier transform	adsorption	0.01	0.1-10	monocrotophos	50 days	91

CHIT-chitosan; GCE-glassy carbon electrode; HGNs-hollow gold nanospheres; MWCNTs-multi-walled carbon nanotubes; PB-Prussian blue; PPy-polypyrrole; SPCE-screen printed carbon electrode.

Table 1. Analytical performances of some nanostructured acetylcholinesterase based sensors for OPs determination.

http://dx.doi.org/10.5772/58310

5. Conclusion

This review addresses the recent trends in the development of nanomaterials based electrochemical biosensors for organophosphorus pesticides determination. The included examples demonstrate the great potential of the carbon nanotubes and the gold nanoparticles, as well as of the emerging graphene structures.

Current researches confirm that the adequate combination of nanomaterials, biological recognition events, and efficient electronic signal transduction result in biosensors with improved analytical performances, appropriate for the high sensitive determination of OPs, among other.

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