Electrochemical Biosensors for the Detection of Pesticides

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Abstract: Biosensors have been developed for the detection of pesticides using integrated enzymes, antibodies, cell and DNA-based biosensors. Enzymatic determination of pesticides is most often based on inhibition of the activity of selected enzymes such as cholinesterase, acid phosphatase, ascorbate oxidase, acetolactate synthase and aldehyde dehydrogenase. Enzymatic Biosensors were developed using various electrochemical signal transducers and different electrodes. Various immobilization protocols used for the formation of a biorecognition interface are also discussed: In addition, techniques of regeneration, single amplification, and miniaturization are evaluated for the development of immunosensor. Both batch and flow-injection analyses with enzyme biosensors are most intensively developed. It included that, in the future, compact, disposable and portable devices especially designed for *in-field* analysis with high sensitivity, selectivity; development of arrays and multiple sensors will continue another area of intensive research for biosensors.

Keywords: Pesticides, electrochemical biosensors, detection.

1. INTRODUCTION

Pesticides (herbicides, fungicides, insecticides) are widely used in the agriculture and industry around the world due to their high insecticidal activity [1, 2]. The presence of pesticide residues and metabolites in food, water and soil currently represents one of the major issues for environmental chemistry. Pesticides are, in fact, among the most important environmental pollutants because of their increasing use in agriculture [3-5].

Among the pesticides, organophosphorus and carbamate insecticides form an important class of toxic compounds; their toxicity is based on the inhibition of acetylcholinesterase (AChE). Organophosphate and carbamate pesticides toxicity can vary considerably, depending on the chemical structure of the pesticide [6, 7]. Many methods are available for pesticide detection: chromatographic methods, such as gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). These methods are very sensitive and reliable but present strong drawbacks such as complex and time-consuming treatments of the samples, i.e. extraction of pesticides, extract cleaning, solvent substitution, etc. [8-12]. Moreover, they can only be performed by highly trained technicians and are not convenient for on-site or on in-field detection.

Biosensors are potentially useful as they detected pesticides quickly and have been active in the research area for some years. Biosensors have been defined as analytical devices which tightly combine bio-recognition elements with physical transducers for detection of the target compounds [13-15] (Fig. **1**). The biological recognition element (enzyme, antibody, microorganism or DNA), in this case, the biosensor is based on a reaction catalyzed by macromolecules, which are present in their biological environment, have been isolated previously or have been manufactured. Thus, a continuous consumption of substrate(s) is achieved by the immobilized biocatalyst incorporated into the sensor: transient or steady-state responses are monitored by the integrated detector.

The transducer part of the sensor serves to transfer the signal from the output domain of the recognition system to, mostly, the electrical domain. The transducer part of a sensor is also called a detector, sensor or electrode, but the term transducer is preferred, to avoid confusion. Example of electrochemical transducers (Potentiometry, amperometry, voltammetry, surface charge using field effect transistors (FETs), and conductometry) which are often used to measure the output signal from the biorecognition domain.

1.1. Sensing Mode

1.1.1. Amperometry

Amperometry is based on the measurement of current resulting from the electrochemical oxidation or reduction of an electroactive species. It is usually performed by maintaining a constant potential at a Pt-, Au- or C-based working electrode or an array of electrodes with respect to a reference electrode (two measuring electrode system without auxiliary electrode), if the current are low (10^{-9} to 10^{-6} A).

Amperometric immunosensors detect the concentration-dependent current, generated when an electroactive species is either oxidized or reduced at the electrode surface to which *Ab-Ag* binds specifically, it is held at a fixed electrical potential. The current is directly proportional to specific *Ab-Ag* binding. The current and bulk concentration of the detecting species can be approximated as:

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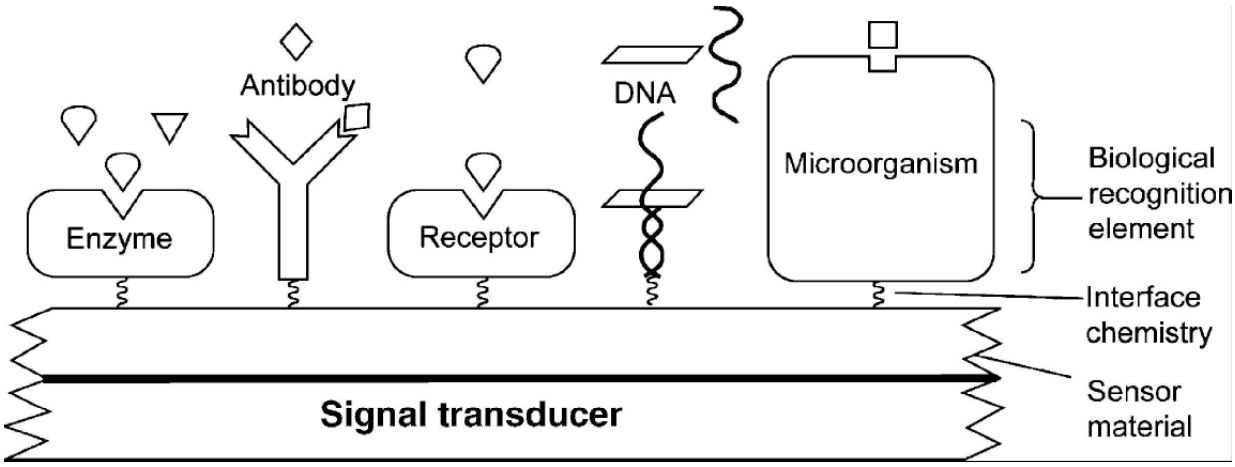


Fig. (1). Schematic representation of biosensors. (*Anal. Chim. Acta*, 2006, 568, 221).

$$I = Z\,F\,k_m\,C^* \quad (1)$$

where I is the current to be measured, Z and F are constants, k_m is the mass transfer coefficient and C^* is the bulk concentration of the detecting species.

1.1.2. Potentiometry

Potentiometric measurements involve the determination of potential difference between both an either indicator and a reference electrode or two reference electrodes separated by a permselective membrane, when there is no significant current flow between them. The most common potentiometric devices are pH electrodes; several other ions (F^-, I^-, CN^-, Na^+, K^+, Ca^+, NH_4) or gas- (CO_2, NH_3) selective electrodes are available.

Potentiometric immunosensors are based on measuring the changes in potential induced by the label used, which occur after the specific binding of the *Ab-Ag*. They measure the potential across an electrochemical cell containing the *Ab* or *Ag*, usually by measuring the activity of either a product or a reactant in the recognition reaction monitored. The measured potential is given by the Nernst equation:

$$E = \text{constant} \pm \frac{RT}{nF} \ln a \quad (2)$$

where E is the potential to be measured, R, T, F are constants, n is the electron transfer number, and a is the relative activity of the ion of interest.

1.1.3. Surface Charge Using Field-Effect Transistors (FETs)

Field effect transistor (FET) and particular ion sensitive FETs (ISFET), have to be presented as a basis for biosensor developments. The main part of an ISFET is ordinary metal oxide silicon FET (MOSFET) with the gate electrode replaced by an ion selective membrane, a solution and a reference electrode. The nature of the membrane /insulator will, then, give the ion specificity of the sensor (pH, NH_3). The pH sensitive IFSETs are the most widely used sensors for the biosensor developments, with a large range of possible insulators (SiO_2, Al_2O_3 and Ta_2O_5) [16-18] and enzyme labels. When such ISFETs are coupled with a biocatalytical or biocomlpexing layer, they become a biosensor, and are usually called either enzyme (ENFETs) or immunological (IMFETs) field-effect transistors.

1.1.4. Conductometry

The principle of the detection is based on the fact that many biochemical reactions in solution produce changes in the electrical resistance (reciprocal conductance). Conductance measurements involve the resistance determination of a sample solution between two parallel electrodes. Many enzyme reactions, such as that of urea and many biological membrane receptors may be monitored by ion conductometric or impedimetric devices, using interdigited microelectrode [19, 20]. In an immunosensor, there is an overall electrical conductivity of the solution and capacity alteration due to the *Ab-Ag* interaction at the electrode surface.

2. BIOSENSORS

Biosensors and bioanalytical methods appears well suited to complement, standard analytical methods for a number of environmental monitoring applications. The definition for a biosensor is generally accepted in the literature as a self contained integrated device consisting of a biological recognition element (enzyme, antibody, receptor, DNA or microorganism) which is interfaced to a chemical sensor (i.e., analytical device) that together reversibly respond in a concentration-dependent manner to chemical species. The use of biosensors for environmental applications has been reviewed in considerable detail [21]. Different recommendations were postulated for defining and describing the characteristic effect on biosensors performance. Some properties and characteristic behaviours of ideal biosensors were evaluated, in accordance with standard IUPAC protocols or definition [22-24]. Which include selectivity, response time, linear range, limit of detection, reproducibility, stability and lifetime.

2.1. Enzyme-Based Biosensor

Enzymes are organic catalysts produced by the living cell that act on substances called substrates. Like all other catalysts, enzymes only catalyse thermodynamically feasible reactions. The enzyme-based sensors measure the rate of the enzyme-catalyzed reaction as the basis for their response, any physical measurement which yields a quantity related to this rate can be used for detection. Several procedures have been devised for the monitoring of the activity of an enzyme using electrochemical transducers. The assessment of this activity usually takes place by the direct measurement of

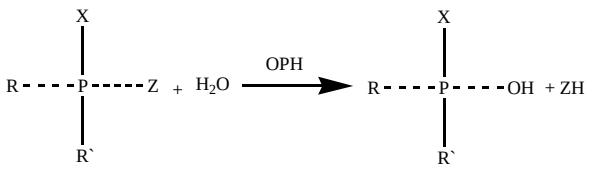
electroactive products or co-substrates involved in the enzymatic reaction. It is possible to realize this monitoring indirectly also using synthetic mediators that favour the transfer of electrons between the electroactive species and the electrode. These procedures are used also in biosensors.

Enzyme immobilization on the transducers is an indispensable step in the development of biosensors. The simplest form of immobilization is to dissolve the enzyme in the buffer solution, depositing it on the electrode surface and covering it with a dialysis membrane. Other immobilization techniques are based on the physical entrapment of the enzyme, inside a synthetic gel layer (formed by the copolymerization of acrylamide and bisacrylamide) or a chemical bond between the enzyme and a membrane or an organic or inorganic support or directly to the transducer (made of Pt, Au, C etc.). The enzyme can be immobilized also by crosslinking with an inert protein with gluteraldehyde and forming insoluble macromolecular aggregates. Different immobilization issues have also been discussed in the literature [25-30]. Many biosensors (enzyme-based biosensor) which are used for pesticide detection are catalytic activity based or are the reaction inhibition, of several enzymes in the presence of pesticides.

2.1.1. Enzymatic Biosensors for Direct Detection of Pesticides

Organophosphorus hydrolase (OPH) is an organophosphotriester hydrolyzing enzyme; the enzyme has broad substrate specificity and is able to hydrolyze a number of organic phosphorus (OP) pesticides such as paraoxon, parathion, coumaphos, diazinon, dursban, etc., as in equation (3). Organophosphorus acid anhydrolase catalyzed hydrolysis of OP compounds generates two protons as a result of the cleavage of the P-O, P-F, P-S or P-CN bonds and an alcohol, which in many cases is chromophoric and/or electroactive. The resulting hydrogen ion can be followed by potentiometry. Organophosphorus hydrolase can be integrated with an amperometric transducer to monitor the oxidation or reduction current of the hydrolysis products (equation 3). Several review articles on integrated organophoshoshate hydrolase enzyme for identification of different classes of pesticides (e.g., carbamates and organophosphates) were published [31-33].

Organophosphorus hydrolase enzyme was utilized as a biosensor for detection of paraoxon and parathion [34]. The transducer structure of the sensors, chip consists of a pH-sensitive capacitive electrolyte-insulator-semiconductor (EIS) structure that reacts towards pH changes caused by the OPH-catalysed hydrolysis of the organophosphate compounds (according to the following equation) (3)

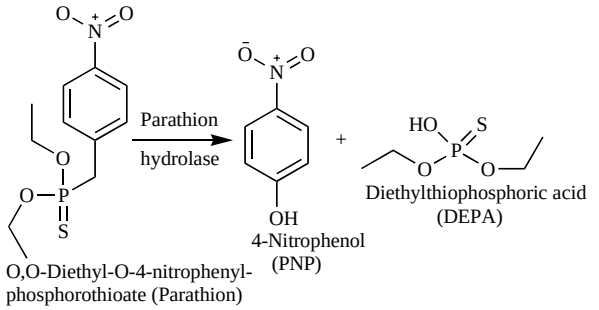


(3)

where, *X* is oxygen or sulfur, *R* is an alkoxy group ranging in size from methoxy to butoxy, *R`* is an alkoxy or phenyl group and *Z* is a phenoxy group, a thiol moiety, a cyanide or a fluorine group.

Biosensors for organophosphate pesticide, containing fluorine were fabricated using the enzyme organophosphorus acid anhydrolase (OPAA) [35]. Batch-mode and stop-flow assays were carried out for the detection of di-isopropyl fluorophosphate and the detection limits were found to be 20 and 12.5 μM in batch-mode and stop-flow assays, respectively. Linear potentiometric responses were obtained for up to 500 mM.

An amperometric enzyme biosensor for the direct measurement of parathion was developed [36]. The biosensor was based on parathion hydrolase. The enzyme was immobilized on a carbon electrode, catalyses the hydrolysis of parathion to form p-nitrophenol (according the following equation) (4), which was detected by its anodic oxidation. The detection limit was less than 1 ng/ml.



(4)

Another example based on the same principle was reported [37]. The detection limits were 15 and 20 nM for parathion and paraoxon, respectively.

Organophosphate pesticides in water were determined using a flow injection amperometric biosensor which incorporated, immobilized organophosphorus hydrolase on activated aminopropyl glass beads with an electrochemical flow through a detector containing a carbon paste working electrode, Ag/AgCl reference electrode and a stainless steel counter electrode [38]. The amperometric response was linear up to 120 and 140μM for paraoxon and methyl parathion, respectively, with detection limits of 20nM for both analyte.

A novel dual amperometric/potentiometric biosensor chip with the immobilized enzyme OPH has been developed and examined for the detection of organophosphorus pesticide [39]. The amperometric and potentiometric transducers of the biosensor chip have been prepared by means of thin-film techniques. Different groups of organophosphorus pesticides, like paraoxon, parathion, dichlorvos and diazinon down to the lower μM concentration range were detected.

A dual-transducer flow-injection biosensor detection system for monitoring organophosphorus (OP) neurotoxins was described [40]. The biosensor was based on OPH. The enzyme catalyses the hydrolysis of parathion to form oxidizable p-nitrophenol and organic acid. The potentiometric biosensors respond favorably to all OP compounds, reflecting the pH changes associated with the OPH activity, and the amperometric devices display well-defined signals only towards OP substrates, (pesticides) liberating the oxidizable p-nitrophenol product. Table **1** summarizes the most common enzymatic biosensor for direct detection of pesticides.

2.1.2. Biosensors Based on Inhibition of Enzyme Activity

Enzymatic determination of pesticides is most often based on inhibition of the activity of selected enzymes such

Table 1. Enzymatic Biosensors for Direct Detection of Pesticides

ANALYTE	ENZYME	DETECTION LIMIT	SYSTEM	REFS.
Di-isopropyl Fluorophosphates	OPAA	20 and 12.5 μM for batch and flow injection	Amperometry	[35]
Parathion	Parathion hydrolase	1 ng/ml	"	[36]
Parathion / Paroxon	Parathion hydrolase	15/ 20 nM	"	[37]
Paraoxon / methyl parathion	OPH	20nM	"	[38]
organophosphorus neurotoxin	OPH	2μM and 6μ for paraoxon dichlorvos, respectively (potentiometry) and 70nM for paraoxon (amperometry)	Amperometry/potentiometry	[40]

OPAA, organophosphorus acid anhydrolase; OPH, organophosphorus hydrolase; OP, organophosphorus

as cholinesterase, acid phosphatase, tyrosinase, ascorbate oxidase, acetolactate synthase and aldehyde dehydrogenase. Such compounds can form stable complexes with some enzymes. This is because those pesticides have a shape that resembles the shape of the substrate, thus blocking the active center of enzyme and inhibiting its activity. This inhibition is independent of the presence of substrate. Enzymatic biosensors were developed using various electrochemical signal transducers, different methods of enzyme immobilization and various measuring methodologies. Application of single-use screen-printed biosensors in batch measurements and flow-injection analysis with enzyme biosensors, are the most intensively developed procedures. Enzyme inhibition by pesticides was used for measuring purpose using the electrochemical sensors and several review articles have been published [41-43].

2.1.2.1. Cholinesterase Enzymes

2.1.2.1.1. Mono-Enzymatic Biosensors

When using acetylcholine (ACh) or butyrylcholine (BuCh) as substrate, the reaction products are choline (Ch) and the corresponding organic acid (Fig. **12** first equation). Since choline is not electrochemically active, the change of enzyme activity is detected by the pH change variation due to the acid production at the surface of the biosensor. In this case, the electrochemical method of choice is a potentiometric one. When artificial substrates, such as acetylthiocholine (ATCh) or butyrylthiocholine (BuTCh) are used, the products of the reaction are thiocholin (TCh) and an organic acid (according to the following reactions (5 and 6). Thiocholine can be oxidized anodically using platinum electrodes or modified electrodes. Recently, a review article on cholinesterase biosensors from basic research to its practical applications was published [44].

$$\text{Acetythiocholine or Butyrylthiocholine} + H_2O \xrightarrow{ChE} \text{Thiocholine} + \text{Organic acid} \quad (5)$$

$$\text{Thiocholine} \xrightarrow{oxidation} \text{dithio-bis-choline} + 2e^- + 2H^+ + 2CI^- \quad (6)$$

Potentiometric biosensors based on butyrylcholinesterase were developed by co-reticulation of the enzyme with glutaraldehyde on an electropolymerized polyethylenimine film at the electrode surface [45]. The butyrylcholinesterase-electrode was tested as a biochemical sensor for the detection of an organophosphorus pesticide, trichlorfon, based on enzyme inhibition. The enzyme electrode showed a detection limit for trichlorfon of < 0.1μM.

Cholinesterase sensors based on glassy carbon and planar epoxy graphite electrodes, modified with processed polyaniline were developed to examine pesticide detection [46]. The modification of electrode surface with polyaniline provides high operational stability and sensitivity towards the pesticides investigated. The detection limits found, (coumaphos, 0.002; trichlorfon, 0.04; aldicarb, 0.03; methiocarb, 0.08 mg/l) made it possible to detect the pollutants in the waters on the level of limited threshold levels without sample preconcentration.

The biosensor methodology was employed to analyze carbaryl directly inside the tomato, without any previous manipulation [47]. In this case, the biosensor was immersed in the tomato pulp (Fig. **2**), which had previously been spiked with the pesticide for 8 min, removed and inserted in the electrochemical cell. A recovery of 83.4% was obtained, showing very low interference of the matrix constituents. The measurements were carried out using an amperometric biosensor technique based on the inhibition of acetylcholinesterase activity due to carbaryl adsorption and a HPLC procedure. The analytical curve obtained in pure solutions showed excellent linearity in the range of 5.0×10^{-5} to 75×10^{-5} mol/l range.

An electrodeposited sub-layer of gold nanoparticles was found to enhance the adsorption and stabilization of AChE on a planar gold electrode surface [48]. The enzyme-modified electrode sensor was utilized for the sensitive electrochemical detection of thiocholine at the gold surface after hydrolysis of acetylthiocholine by the immobilized enzyme. In the absence of the nanoparticle layer, the sensor response to acetylthiocholine was significantly reduced and the utility of the electrode was limited. The ability of the nanoparticle-based (Fig. **3**) sensor to reliable measure concentrations of the organophosphate pesticide carbofuran at nM concentrations was demonstrated by monitoring the inhibition of the hydrolysis of acetylthiocholine.

Sol-gel-derived silicate network assembling gold nanoparticles (AuNPs-SiSG) provides a biocompatible microenvironment around the enzyme molecule to stabilize its biological activity and prevent them from leaking out of the interface was constructed [49]. Typical pesticides such as monocrotophos, methyl parathion and carbaryl were selected for pesticide sensitivity tests. The proposed electrochemical

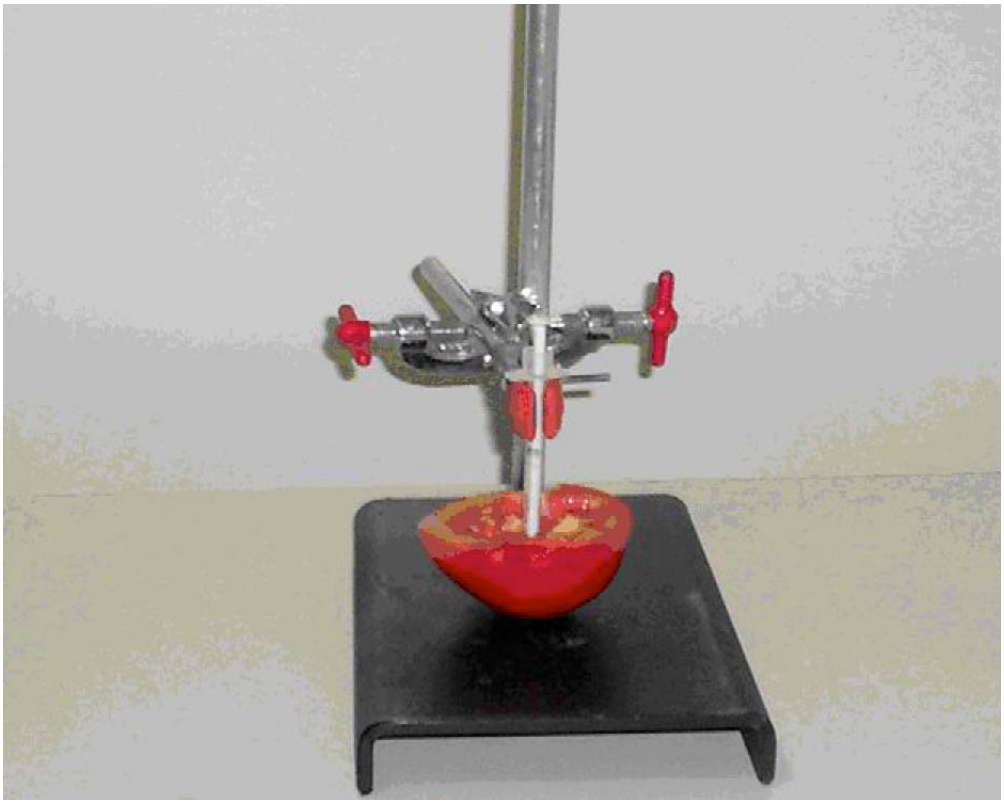


Fig. (2). Photograph of the experimental set-up for immersion of the biosensor in the Tomato "in natural", spiked with carbaryl. (*Sensors and Actuators* B129, 2008, 40).

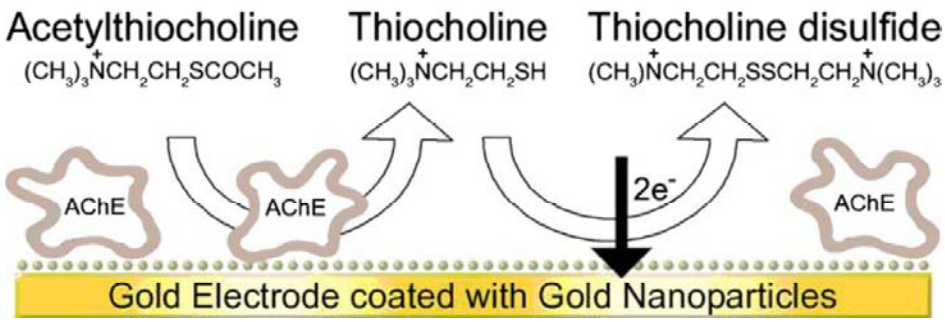


Fig. (3). Schematic diagram of the enzymatic reaction at the gold nanoparticle-coated AChE electrode. (*Electrochemistry Communications*, 2007, 9, 935).

pesticide sensitivity test exhibited high sensitivity, desirable accuracy, low cost and simplified the procedures.

One-step electrochemical deposition of gold nanoparticles in chitosan hydrogel onto a planar gold electrode (Fig. **4**) was used to create a favorable surface for the attachment of the enzyme AChE [50]. The proposed method for rapid determination of malathion was established based on the chemisorption / desorption process of thiocholine used as an indicator. Under the optimal conditions, the decrease in response was proportional to the concentration of malathion from 0.1 - 20 ng/ml, with detection limit of 0.03 ng/ml.

For amperometric detection of cholinesterase activity, both the substrates acetylcholine and acetylthiocholine have been extensively used. The latter is preferable because it avoids the use of another enzyme, choline oxidase, which is usually used with acetylcholine. However, the amperometric measure of thiocholine, produced by the enzymatically catalysed hydrolysis of acetylthiocholine, has proved to be difficult at classic electrode surfaces due to the high over potential needed as well as the possible problems of surface passivation [51]. To overcome this problem other electrodes or chemical modifiers have been used.

Immobilization of AChE enzyme on multiwall carbon nanotubes [52] and multiwall carbon nano-chitosan [53] was proposed and thus a sensitive, fast and stable amperometric sensor for quantitative determination of organophosphorous insecticide was developed. Under optimal conditions the inhibition of triazophos was proportional to its concentration in two ranges, from 0.03 to 7.8 and 7.8 to 32 μM with a detection limit of 0.01 μM [53].

An amperometric biosensor based on the adsorption of the AChE enzyme on screen printing electrodes [54] and SPE coated with a Nafion layer [55] were investigated. The sensor SPE [54] was used to detect the inhibitory effects of

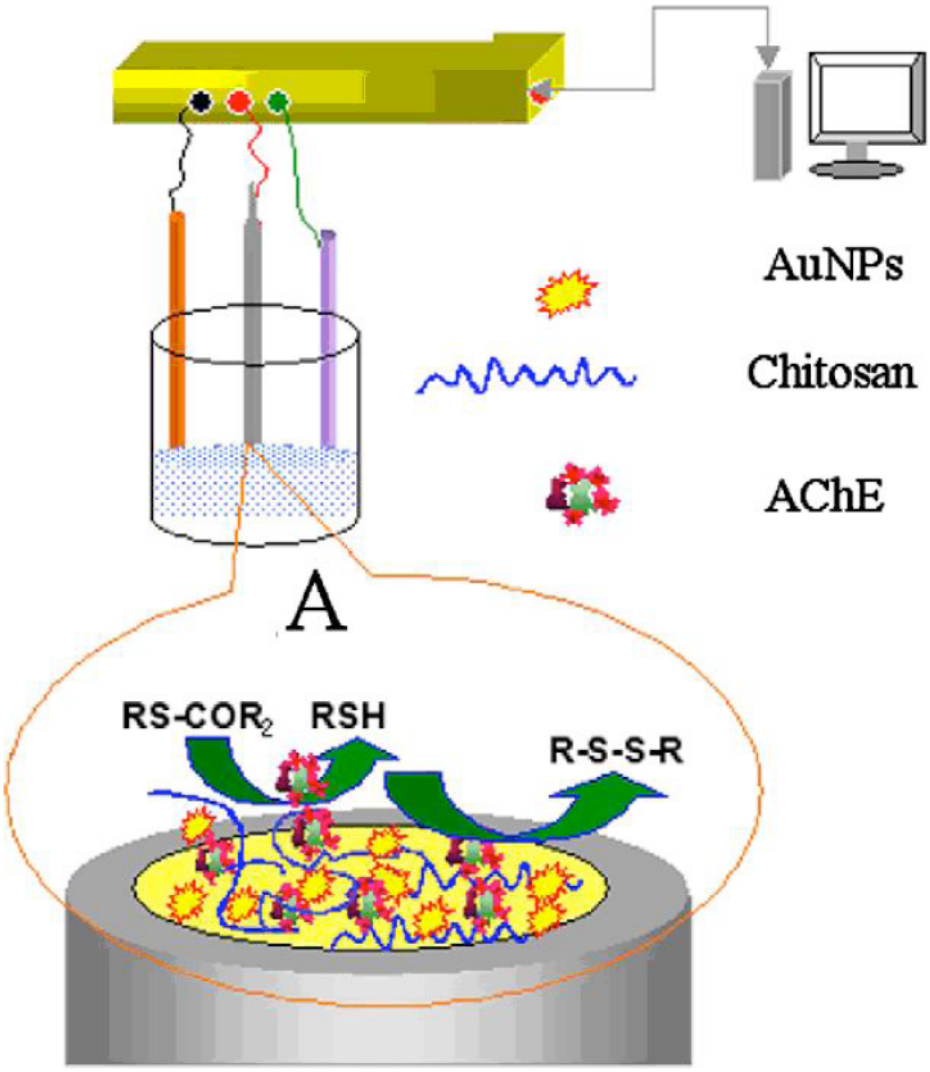


Fig. (4). Mechanism of constructed biosensor based on one-step electrodeposition. (A) Megascopic interface of AChE/CHIT–GNPs modified gold electrode. (*J. Electroanalyt. Chem*., 2007, 605, 53).

organophosphorus and carbamate insecticides on acetylcholinesterase, and more particularly on chlorpyrifos ethyl oxon. The detection limits were found to be 0.35 and 0.15μM for trichlorfon and coumaphos, respectively [55]. Figs. (**5** and **6**) show the diagram of the integrated two and three screen-printed electrodes.

A screen-printed biosensor for the detection of pesticides in water-miscible organic solvents was described based on the use of p-aminophenyl acetate as acetylcholinesterase (AChE) substrate [56] (Fig. **7**). The oxidation of p-aminophenol, product of the enzymatic reaction, was monitored at 100 mV (vs. Ag/AgCl screen-printed reference electrode). The sensor showed good characteristics when experiments were performed in concentrations of organic solvents below 10%. No significant differences were observed when working with 1 and 5% acetonitrile in the reaction media. Detection limits as low as 19.1 and 1.24 nM for paraoxon and chlorpyrifos ethyloxon respectively, were obtained when experiments were carried out in 5% acetonitrile.

The use of modified electrode surfaces capable of oxidising thiocholine applied at low potentials and without passivation has been proposed. 7,7,8,8- tetracyanoquinodimethane(TCNQ) was used as an electrochemical mediator for thiocholine detection [57]. The detection of N-methylcarbamate insecticides: aldicarb, carbaryl, carbofuran and methomyl were investigated. The LOD were determined with a minimum 10% inhibition, and varied from 1-8nM (0.2-1.5 ppb) by employing the enzyme immobilization through photopolymerization.

Screen-printed electrodes were adopted and modified by depositing TCNQ and prussian blue was developed and tested for detection of anticholinesterase pesticides in aqueous solution and in spiked grape juice [58]. The influence of enzyme source and detection mode on biosensor performance was explored. The slopes of the calibration curves obtained with modified electrodes were increased by two folds and the detection limits of the pesticides were reduced by factors of 1.6 to 1.8 in comparison with the use of unmodified transducers. The biosensors developed made it possible to detect down to 2×10^{-8}, 5×10^{-8}, and 8×10^{-9}M for chloropyrifosmethyl, coumaphos, and carbofuran respectively, in aqueous solution and grape juice.

Cobalt phthalocyanine (Co-phthalocyanine), after its first demonstrated use as thiocholine mediator, remains one of the

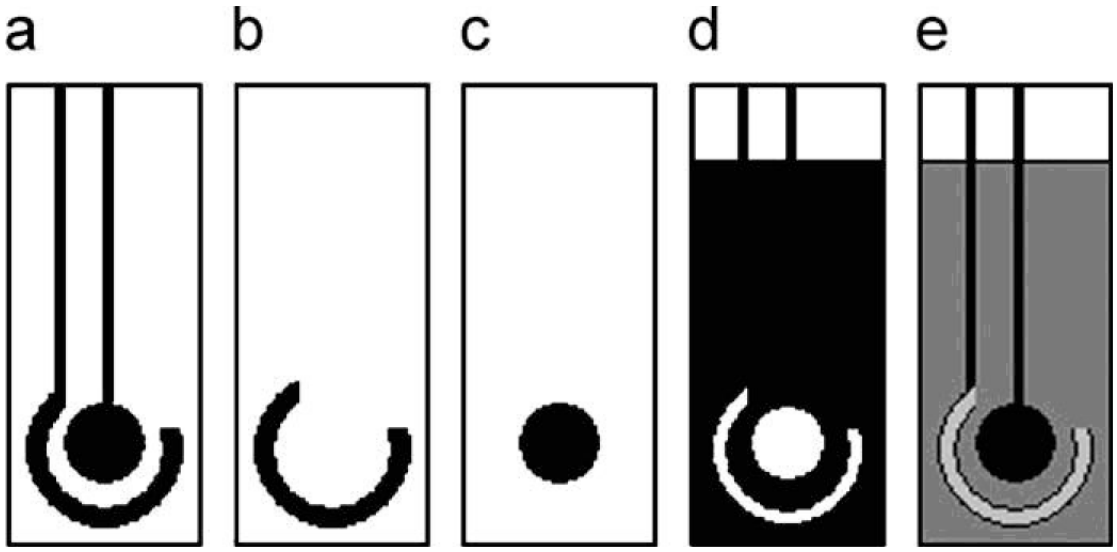


Fig. (5). Design of screens for 2-electrode biosensor: (a) basal track; (b) reference electrode; (c) working electrode; (d) insulation coating; (e) schematic of two-electrode screen-printed sensor. (*Ecotoxicology and Environmental Safety*, 2008, 69, 556).

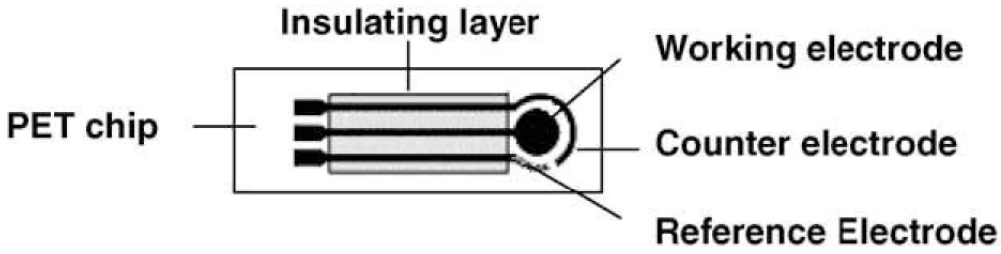


Fig. (6). The diagram of the integrated three screen-printed electrodes. (*Talanta*, 2006, 68, 1089).

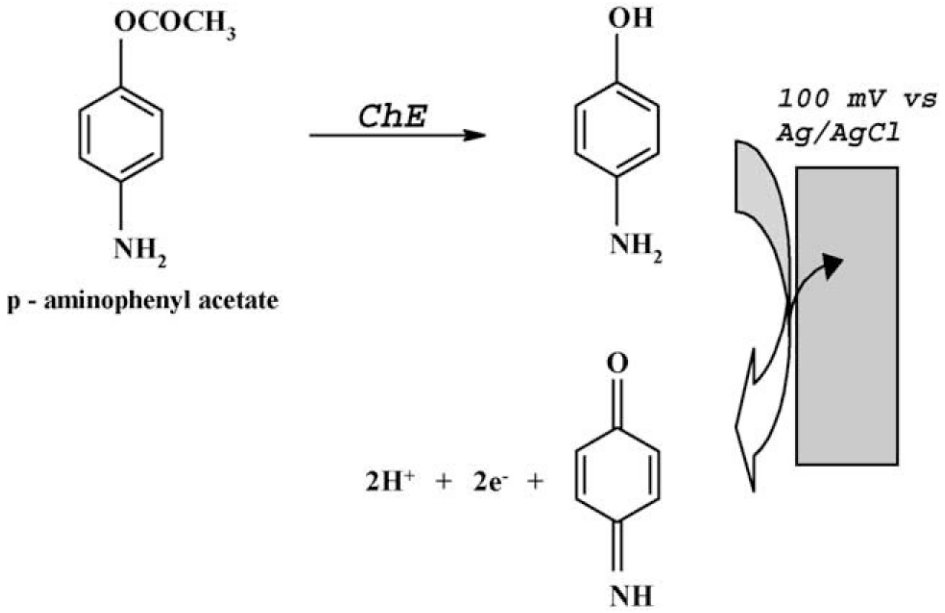


Fig. (7). Mono-enzymatic amperometric ChE biosensor based on p-aminophenyl acetate as substrate. (*Biomolecular Engineering*, 2006, 23, 1).

most used electrocatalysts for this purpose. The best example of the use of such mediator, in terms of easiness of production and sensitivity towards thiocholine, still remains the bulk-modified Co-phthalocyanine electrode, which has been extensively used for the pesticide detection purpose [59-61] (Fig. **8**).

Prussian blue-modified screen printed electrode (SPE) is one of the most commonly used electrochemical modifier [62]. In a recent comparative study Co-phthalocyanine and Prussian blue-modified screen-printed electrodes has been performed [63] and both the electrodes demonstrated an easiness of preparation together with high sensitivity towards

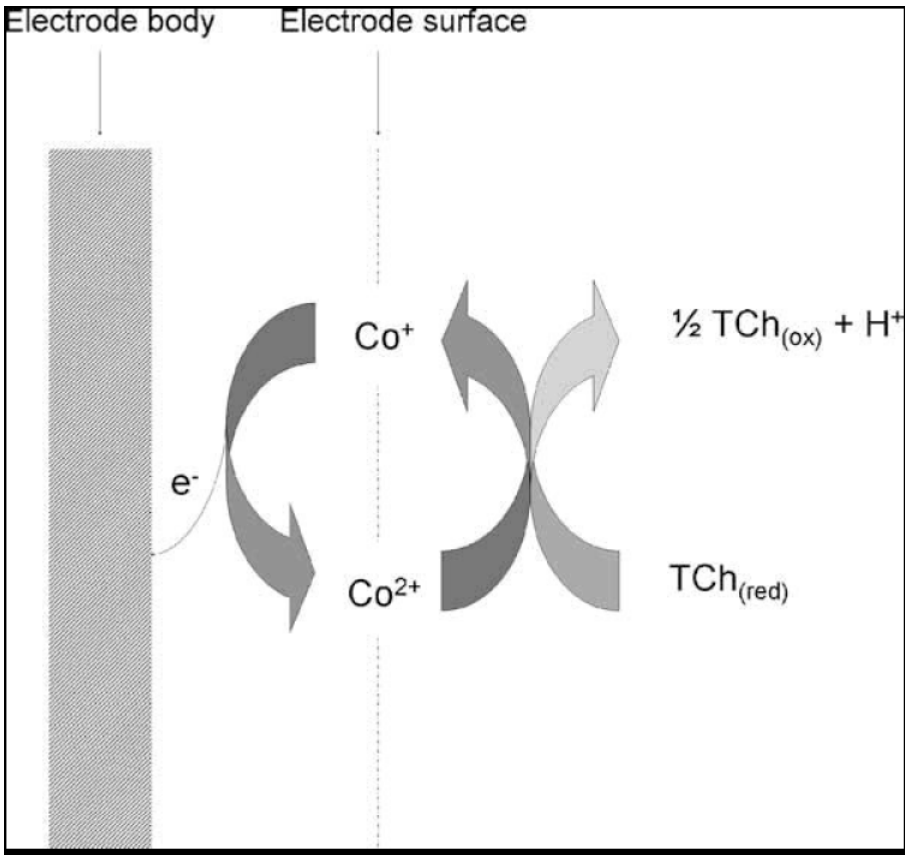


Fig. (8). Schematic representation of the Co-phthalocyanine mediated electrode surface. (*Biosens. Bioelectronics,* 2004, 20, 765).

thicoholine (LOD = 5×10^{-7} and 5×10^{-6}M for Co-phthalocyanine and Prussian blue, respectively) with high potentialities for pesticide measurement [63]. Prussian blue-modified screen-printed electrodes were then selected for successive enzyme immobilization, due to their higher operative stability demonstrated in previous works. AChE and BChE enzymes were used and inhibition effect of different pesticides was studied with both the enzymes. AChE-based biosensors have demonstrated a higher sensitivity towards aldicarb (50% inhibition with 50 ppb) and carbaryl (50% inhibition with 85 ppb) while BChE biosensors have shown a higher affinity towards paraoxon (50% inhibition with 4 ppb) and chlorpyrifos-methyl oxon (50% inhibition with 1 ppb). Real samples were also tested in order to evaluate the matrix effect and the recovery values comprising between 79 and 123% were obtained.

The use of a disposable biosensor, offers some additional advantages such as mass production, possibility for miniaturization and low cost. The disposable biosensors for pesticides were fabricated by immobilizing an enzyme (acetylcholinesterase or butyrylcholinesterase) on to a SPE-epoxy composite layer applied to the conducting copper tracks on a glass fibre substrate [64]. The detection limits were 0.2 and 0.6nM and RSD were 7- 9 % for carbofuran and paraoxon respectively. The recoveries of 0.001-10μM-carbofuran and paraoxon from tap water and orange juice were quantitative.

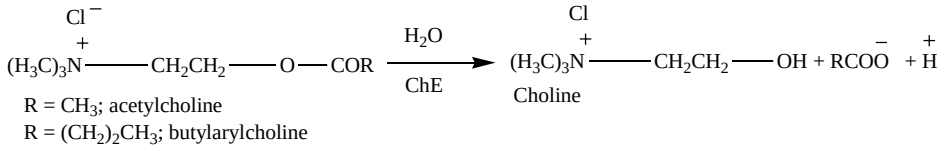
Another disposable cholinesterase biosensor based on SPEs was assembled for organophosphorus pesticides [65-69] by which the lowest amount 1ppb of chlorpyrifos-ethyl oxon can be detected [65].

A simple, reproducible and stable amperometric AChE-based bioelectrode in organic solvents medium was constructed showing good analytical characteristics and appeared to be suitable for the detection of pesticides in the presence of small amount of organic solvent [70]. The inhibition percentage induced by a paraoxon in organic solvent solutions increases in the following sequence: acetonitrile < water < hexane, suggesting that the paraoxon repartition between the organic solvent and the essential water for enzyme activity plays an important role in establishing the analytical and kinetic parameters of the bioelectrode.

The pre-investigated work was presented for the construction of an amperometric biosensor, for highly sensitive detection of organic phosphorus insecticide dichlorvos, based on the inhibition of genetically modified AChE [71]. The biosensor was able to work in the presence of 5% acetonitrile, which was necessary for the extraction of pesticide from the sample. The use of enzymatic biosensor in organic solvent was also reported with good reproducibility [72, 73].

2.1.2.1.2. Bi-Enzymatic Biosensors

In this system, chlolinestrease (ChE) is coupled to a second enzyme choline oxidase (ChO) (equation 7 and 8). In the reaction of oxidation of choline catalyzed by choline oxidase, oxygen is consumed during the reaction and hydrogen peroxide is produced. Hence, change of concentration of one of these can be the basis for the bienzymatic response. Oxygen, detection is achieved by Clark electrodes and H_2O_2 with platinum, graphite or screen print electrodes or other electrodes [74].



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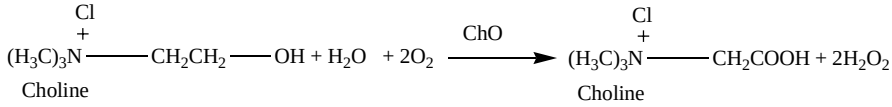

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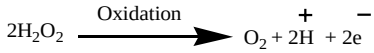

(9)

A disposable carbon nanotube-based biosensor was successfully developed and applied to the detection of OP pesticides and nerve agents [75]. The biosensors using acetylcholinesterase (AChE)/choline oxidase (CHO) enzymes provided a high sensitivity, wide linear range and low detection limits for the analysis of OP compounds. Such characteristics may be attributed to the catalytic activity of carbon nanotubes to promote the redox reaction of hydrogen peroxide produced during AChE/CHO enzymatic reactions with their substrate, as well as the large surface area of carbon nanotube materials.

A new design of an enzyme biosensor based on AChE and ChO immobilized on the supported monomolecular layer composed of poly (amidoamine) of the fourth generation mixed with 1-hexadecanethiol was developed [76]. The resulting enzymatic activity, measured amperometrically, was substantially depressed in the presence of the organophosphate pesticide dimethyl-2, 2-dichlorovinylphosphate (DDVP, Dichlorvos), carbamate pesticides carbofuran and carbamate drug eserine. The detection limits (1.3×10^{-3}, 0.01 ppb and 0.03 for DDVP, carbofuran, and eserine respectively).

Acetylcholinesterase and choline oxidase were co-immobilized on poly (2-hydroxyethyl methacrylate) membranes to construct a biosensor for the detection of anti-cholinesterase compounds [77]. Enzyme immobilized membrane was used in the detection of anti-cholinesterase activity of aldicarb (AC), carbofuran (CF) and carbaryl (CL), as well as two mixtures, (AC + CF) and (AC + CL) were detected. The total anti-cholinesterase activity of binary pesticide mixtures was found to be lower than the sum of the individual inhibition values.

An amperometric biosensor for pesticides detection was prepared using bienzymes (AChE /ChO) and acetylcholine as substrate. Choline oxidase was adsorbed on to the graphite working electrode [66]. The biosensor was employed to determine acetylcholinesterase inhibiting pesticides in fruit and vegetables using acetylcholine as a substrate. The analysis was carried out by incubating the prepared extract with borate buffer of pH 9 containing 0.1M-KCl and acetylcholinesterase for 10 min. Acetylcholine was then added and after 2 min the concentration of choline was measured using the biosensor at 700 mV vs. SCE. The method was calibrated with carbofuran. Calibration graphs were linear from 0.01-0.4 μ mol/l and the detection limit was 2 μg/l.

2.1.2.1.3. Tri-Enzymatic Biosensors

Peroxidase (POD) may be added to the bi-enzyme system to build a tri-enzyme device (equation 9). The generation of H_2O_2 as a product of the second reaction provokes a potential change in the electrode. This change is due to the bioelectrocatalysis of peroxide, where POD is regenerated without the presence of a mediator. Direct electron transfer to POD takes place on the electrode causing the potential change. This potential shift is proportional to the H_2O_2 concentration and to the activity of the cholinesterase.

The sensor was based on the ability of organophosphorus pesticides to inhibit the catalytic activity of cholinesterase [78]. Immobilized peroxidase, functioning as a molecular transducer, catalyses the electroreduction of H_2O_2 by direct electron transfer. The sensing element comprises of carbon-based electrode covered by a layer of three co-immobilized enzymes, *viz*, cholinesterase, choline oxidase and peroxidase. Glutaraldehyde was used as a binding agent. Measurement of electrode activity takes 3-5 min. Trichlorfon could be determined in the nM concentration range with a detection limit of 5nM.

2.1.3. Acid Phosphatase

Biocatalytic hydrolysis of glucose-6-phosphate in the presence of acid phosphatase (AP) is reversibly inhibited by organophosphorus and carbamate pesticides. Amperometric detection of this inhibition requires a bienzymatic system with glucose oxidase (GOD) according the following reactions, and final measurement of hydrogen peroxide:

$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{AP} \text{Glucose} + \text{inorganic phosphate} \quad (10)$$

$$\text{Glucose} + O_2 \xrightarrow{GOD} \text{Gluconolactone} + H_2O_2 \quad (11)$$

Both enzymes were immobilized on separate membranes using the polyazetidine prepolymer as an immobilizing agent [79], and amperometric determination of the H_2O_2 at Pt electrode.

Two amperometric bienzyme biosensors were described [80] for determining organophosphorus and carbamic acid pesticides, namely: (i) a classical biosensor in which purified AP and GOD were immobilized on to separate membranes and the membranes were attached to a commercial H_2O_2 sensor and (ii) a hybrid biosensor in which GOD was spread on to potato tissue and the potato tissue was attached to the commercial H_2O_2 sensor. The detection limits were 0.5 - 3 and 0.5 - 1.5 μg/l for the classical and hybrid biosensors, respectively. The detection limits for a carbamic acid pesticide (aldicarb) were 40 μg/l for both types of biosensor and the linear range was 46 -125 μg/l. The hybrid biosensor ex-

hibited a longer shelf life and a better reliability than the classical biosensor.

Chemometric methods for the development of a biosensor system and the evaluation of inhibition studies with solutions and mixtures of pesticides and heavy metals were developed [81]. The system consisted of three pH electrodes, and the ion sensitive area of each electrode was covered with a cellulose acetate membrane incorporating acetylcholinesterase, alkaline phosphatase or acid phosphatase; the substrates were acetylcholine chloride, alpha-D-glucose-1-phosphate disodium salt and 4-nitrophenylphosphate for the three enzymes, respectively. The relative inhibition of each test substance was obtained by potentiometrically measuring the change in enzyme activity after immersion for 1 h in the test solution.

2.1.4. Tyrosinase

Tryosinase (polyphenol oxidase) catalyzes the oxidation of monophenol to o-diphenols and further to o-quinones:

$$\text{Monophenol} + O_2 \xrightarrow{\text{Tyrosinase}} \text{Quinone} + H_2O \qquad (12)$$

The progress of reaction can be followed amperometrically by reduction of quinone.

In several published articles it was demonstrated, that revisable inhibition of tyrosinase can be utilized for determination of various pesticides of different structure [82-89].

A tyrosinase (Tyr) screen-printed biosensor based on the electroreduction of enzymatically generated quinoid products was electrochemically characterized and optimized for determination of carbamates and organophosphorus pesticides [82]. A composite electrode prepared by screen-printing a Co-phthalocyanine modified cellulose-graphite composite on a polycarbonate support was employed as an electrochemical transducer. The Tyr biosensor was prepared by immobilization of enzyme on the composite electrode surface by cross-linking with glutaraldehyde and bovine serum albumin. The results shown that the methyl parathion and carbofuran can lead to competitive inhibition process of the enzyme while diazinon and carbaryl act as mixed inhibitors. Linear relationships were found for methyl parathion (6 - 100 ppb), diazinon (19 - 50 ppb), carbofuran (5 - 90 ppb) and carbaryl (10 - 50 ppb). Analysis of natural river water samples spiked with 30 ppb of each pesticide showed recoveries between 92.50% and 98.50% and relative standard deviations of 2%.

A substrate-bound tyrosinase electrode was used to detect pesticide without substrate standard solution by immobilizing both the enzyme and the substrate on the gold nanoparticles [83]. Tyrosinase was activated by the use of reduced pyrroloquinoline quinone which was covalently bonded with the modified gold nanoparticles, the mechanism being identified with cyclic and differential pulse voltammetry. The sensitivity was enhanced by the use of gold nanoparticles and the tyrosinase activity was maintained and converted into current signals (Fig. **9**).

Triazine pesticides were analysed during their inhibiting on the tyrosinase enzyme when operating in water-saturated chloroform medium [84]. Several triazine (simazine, propazine, terbuthylazine) and benzotriazinic (azinphos-ethyl and azinphos-methyl) pesticides were determined. Recovery trials were also performed in vegetal matrixes (corn, barley, lentils) and the detection limit was 0.5×10^{-9} mol/l.

The use of several designs of amperometric enzymatic biosensors based on the immobilized tyrosinase enzyme (Tyr) for determining dichlorvos organophosphate pesticide was described [85]. The biosensors are based on the reversible inhibition of the enzyme and the chronocoulometric measurement of the charge due to the charge-transfer mediator 1, 2-naphthoquinone-4-sulfonate (NQS). Tryosinase becomes active when reducing the quinone form of the mediator molecule (NQS) to the reactive o-diol form substrate of Tyr (H_2NQS) at the working electrode thus permitting modulation of the catalytic activity of the enzyme and measurement of the inhibition produced by the pesticide. A detection limit of about 0.06 μM was obtained for dichlorvos with entrapment of NQS and Tyr within electropolymerized poly(o-phenylenediamine) polymer, which was the design that proved to have the best analytical performance.

A three electrode system was composed of a glassy carbon electrode modified with tyrosinase immobilized with glutaraldehyde, a Ag/AgCl reference electrode and a Pt wire counter electrode was developed for determination of diazinon in ethanol or dichlorvos in H_2O [86]. 1, 2-naphthaquinone-4-sulfonate (NQS) was converted to a reactive diol, which facilitated its use as a bioelectrocatalyst, with a -150 mV pulse for 10s; a 100 mV oxidative pulse terminated the reaction. The inhibitory effects of diazinon or dichlorvos on enzyme activity were monitored ampermetrically from an analysis of the current decay during the reductive pulse. Detection limits were 5 and 75 μM for diazinon and dichlorvos respectively.

Dimethyl- and diethyldithiocarbamates were determined by their inhibiting effect on the catalytic activity of a tyrosinase electrode. The amperometric inhibition measurements were carried out at -0.2 V vs. Ag/AgCl with a Pt wire auxiliary electrode. The enzyme electrode was prepared by coating a graphite electrode with tyrosinase [87]. The test solution was added to 0.4 mM phenol in reversed micelles and the change in steady-state current was monitored. The reversed micelles were prepared by adding 4% aqueous 0.05M-phosphate buffer of pH 7.4 to 0.1M-dioctyl sulfosuccinate in ethyl acetate. Calibration graphs were linear from 0.2 - 2.2, 4 - 4.4 and 4 - 40 μM for Ziram, Diram and zinc diethyldithiocarbamate, respectively; detection limits were 0.074, 1.3 and 1.7 μM, respectively. Relative standard deviation were 5.5 -8% (n = 10) at the lower limit of the linear range. Recovery was 102% of 3.1 mg/kg Ziram from spiked apple.

A review presented of enzyme-based electrochemical biosensors for the determination of organophosphorus and carbamate pesticides which covers cholinesterase-based biosensors, tyrosinase-based biosensors and other enzyme system was published [88].

A biosensor method for the determination of triazine pesticides based on an inhibition organic phase enzyme electrode (OPEE) was described. The OPEE was developed using a tyrosinase biosensor assembled in the version operating in organic phase and used to determine triazine pesticides by exploiting their power to inhibit the tyrosinase enzyme. The tyrosinase OPEE was also used to test triazine

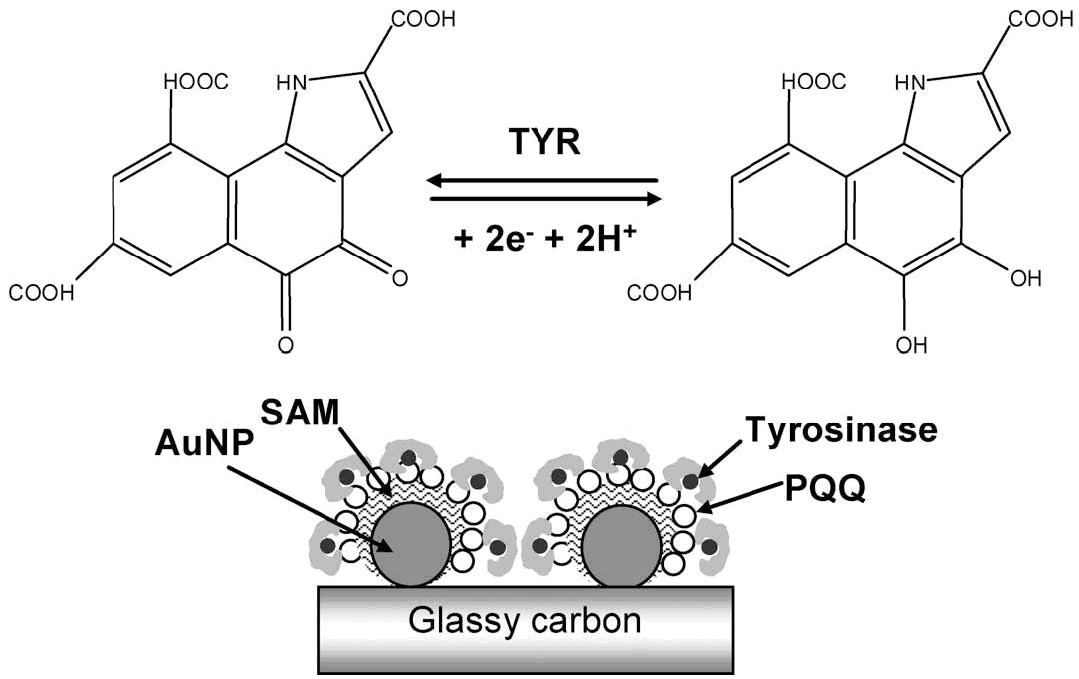

Fig. (9). Schematic diagram of the electrochemical reduction of PQQ and the enzymatic oxidation of $PQQH_2$. (*Sens. and Actuators*, 2008, B133, 1).

recovery from common vegetal samples, obtaining recoveries always >90% [89].

2.1.5. Aldehyde Dehydrogenase

It is known that the dithiocarbamate fungicides inhibit aldehyde dehydrogenase (ADH). In order to produce an amperometric biosensor with this enzyme also a bienzymatic system was designed with diaphorase which operate according to the reactions:

$$\text{Propinoaldehyde} + NAD^+ \xrightarrow{ADH} \text{propionic acid} + NADH + H^+ \quad (13)$$

$$NADH + 2\,Fe\,(CN)_6^{3-} \xrightarrow{Diapharase} NAD^+ + 2\,Fe\,(CN)_6^{4-} + H^+ \quad (14)$$

The changes of hexacyanoferrate (II) concentration are monitored ampetometrically with a Pt electrode [90].

A biosensor for dithiocarbamate fungicides was developed based on the inhibition of ADH [91]. The enzymes, ADH and diaphorase, were immobilized in a poly (vinyl alcohol) film attached to a Pt electrode and covered with a Cellophane membrane. The concentration of fungicide was calculated from the difference in the amperometric signals in the presence and /or absence of the fungicide. The amperometric signals were measured at 100 mV vs. Pt electrode (*viz*. 250 mV *vs*. SCE).

Sensing material prepared from equal volumes of poly (vinyl alcohol) with styrylpyridinium groups and a mixture of aldehyde dehydrogenase and NADH oxidase was spread on a Pt disc working electrode, and the mixture was polymerized. Sensor activity was measured with potassium hexacyanoferrate and NAD^+ in phosphate buffer of pH 7.5 at 30°C [92]. The low solubility of zineb (a dithiocarbamate fungicide) was increased by conversion to the corresponding disodium salt with EDTA disodium salt prior to assay based on its inhibition of the reaction of propionaldehyde with NAD^+ in the presence of aldehyde dehydrogenase. Calibration graphs were linear up to 80 ppm zineb or the corresponding disodium salt and the detection limit was 8 ppb of the disodium salt.

2.1.6. Acetolactate Synthase

Sulfonylurease and imidazolinones are reversible inhibitors of acetolactate synthase (ALS), an essential enzyme for biosynthesis of the branched chain amino acids. Earlier studies indicated the possibility of preparing a biosensor with acetolactate synthase for determination of sulfonylurea herbicides.

Acetolactate synthase was immobilized on to a poly vinyl alcohol membrane and deposited on to the O_2-permeable membrane of an O_2 electrode. Detection was based on the inhibition of an O_2 side reaction of acetolactate synthase by herbicides; decreased O_2 consumption was used as a measure of herbicide concentration according the following reaction, which pyruvate was used as a substrate [93]. The O_2-consuming reaction of the enzyme was monitored for 5 min at 30°C. The biosensor could detect down to 1μM herbicide.

$$\text{Pyruvate} + O_2 \xrightarrow{ALS} \text{peracetate} + CO_2 \quad (15)$$

2.1.7. Ascorbate Oxidase (AOD)

Amperometric detection of some organophosphorus pesticide is based on inhibition of activity of ascorbate oxidase (AOD), which catalysis the following reaction:

$$\text{Ascorbate} + O_2 \xrightarrow{AOD} \text{dehydroascorbate} + H_2O \quad (16)$$

A biosensor for ethyl paraoxon was modified by trapping cucumber tissue (rich in ascorbic acid oxidase) between Teflon and nylon net membranes attached to a Clark-type oxygen electrode [94]. The biosensor was based on the inhibiting action of ethyl paraoxon on ascorbic acid oxidase. A linear response was obtained from 1 to 10 ppm ethyl paraoxon.

2.2. Mode of Measurements

2.2.1. Flow Injection

A mediator-free amperometric biosensor for screening organophosphorus pesticides in flow-injection analysis (FIA) system based on anticholinesterase activity of OPs to immobilized AChE was developed [95]. The enzyme biosensor is prepared by entrapping AChE in Al_2O_3 sol-gel matrix screen-printed on an integrated 3-electrode plastic chip. The detection limit for dichlorvos is achieved at 10 nM in the simulated seawater for 15 min inhibiting time.

Flow injection analyses system to determine malathion in seawater continuously by the biosensor based on the immobilized AChE was studied [96]. Under the optimum condition, the detection limits of the biosensor for malathion in seawater were 1.3 μg/l and 0.05 μg/l before and after preoxidation respectively. A sample containing malathion less than 100 μg/l was measured.

Determination of the organophosphorus pesticides paraoxon, chlorpyrifos oxon, and malaoxon was performed by a method was based on inhibition of AChE and amperometric detection in a FIA with enzymes obtained from different sources (details was given) and immobilized on the surface of platinum electrode within a layer of poly (vinyl alchol) [97]. Determination of the analytes in spiked river water samples by use of the AChE biosensor resulted in recoveries from 50 to 90% for chlorpyrifos oxon at levels of 20 to 40nM, 50 to 100% for paraoxon at 0.6 to 0.8μM, and 140 to 190% for malaoxon at 0.6 to 1.2μM. For example a flow injection system includes both potentiometry and conductometry are shown in Fig. (**10**).

2.2.2. Multi -Electrode Transducers

An amperometric biosensor array was developed [98] to resolve pesticide mixtures of dichlorvos and methylparaoxon. The biosensor array was used in a flow injection system, in order to operate automatically the inhibition procedure. The sensors used were three screen-printed amperometric biosensors that incorporated three different sources of acetylcholinesterase enzymes. The inhibition response triplet was modelled using an Artificial Neural Network which was trained with the mixture solutions that contain dichlorvos from 10^{-4} to 0.1μM and methylparaoxon from 0.001 to 2.5μM. This system can be considered as an inhibition of electronic tongue (Fig. **11**).

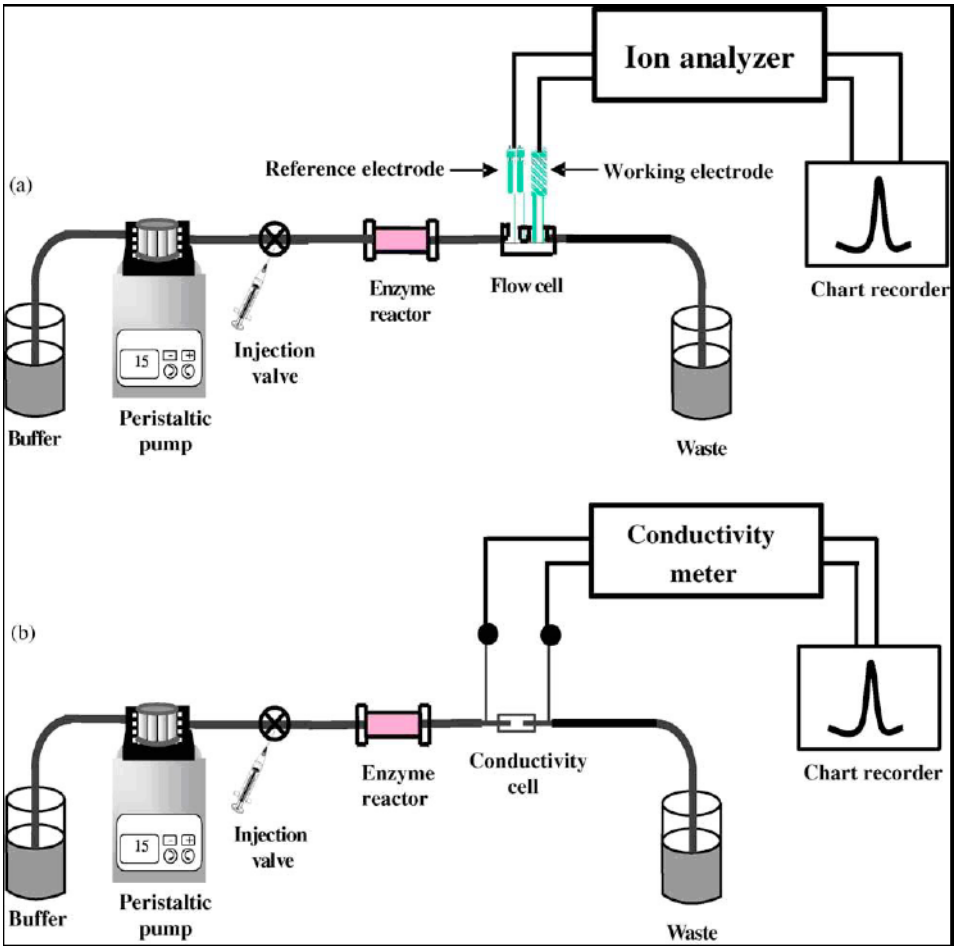

Fig. (10). Schematic diagram showing the flow-injection biosensor systems: (**a**) potentiometric; (**b**) conductimetric. (*Biosen. Bioelectronics*, 2005, 21, 445).

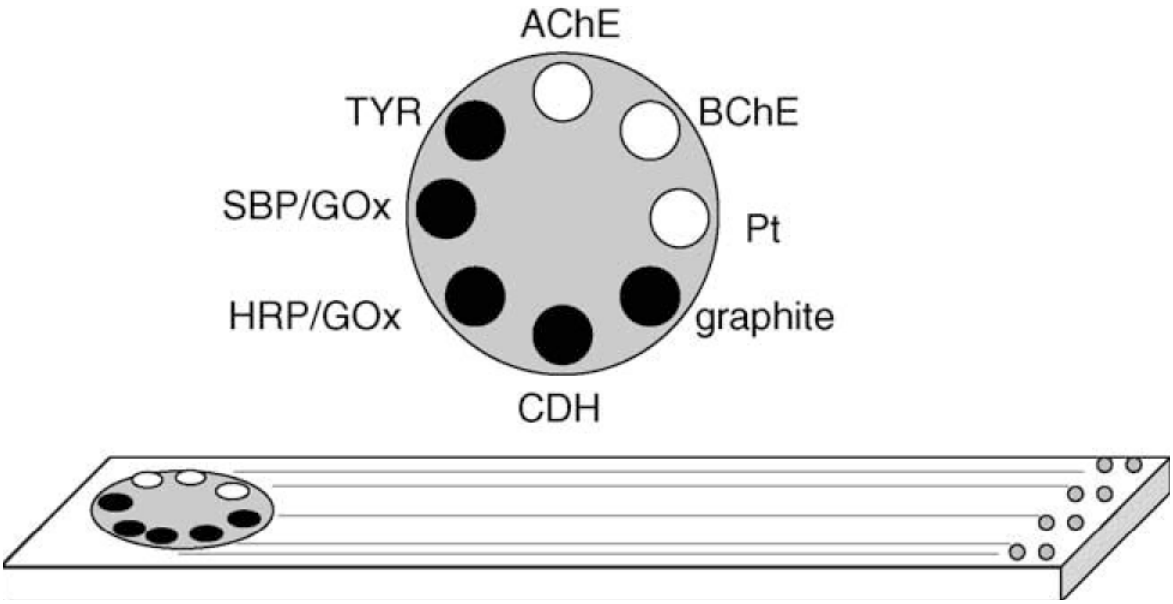

Fig. (11). The construction of the eight-electrode screen-printed array and the illustration of the final distribution of enzymes on the working electrodes, free Pt and graphite electrodes remained uncoated. (*Anal. Chim. Acta*, 2005, 528, 9).

Multielectrode transducers consisting of four pairs of 7,7,8,8-tetracyanodimethane-graphite working electrodes and Ag/AgCl reference electrodes were screen printed. Acetylcholinesterase from *Drosophila melanogaster* and various mutant AChEs were screen-printed onto the working electrodes with photocrosslinkable polyvinyl alcohol solutions and crosslinked under light [99]. Detection and discrimination of binary mixtures of paraoxon, malaoxon and carbofuran cholinesterase-inhibiting insecticides can be assessed by those sensors with prediction errors of 0.9 and 1.6 μg/l, respectively.

2.2.3. Portable Biosensor

The performance of a portable biosensor prototype for the determination of neurotoxic pesticides in water and food samples has been assessed and validated for an *in-field* use [100]. The biosensor is based on the inhibition of the acetylcholinesterase enzyme using screen-printed electrodes and designed potentiostat.

A high sensitive portable biosensor system capable of determining the presence of neurotoxic agents in water was developed [101] (Fig. **12**). The system consists of (i) a screen-printed electrode with AChE immobilized on it, (ii) a self-developed portable potentiostat with an analog to digital converter and a serial interface for transferring data to a portable PC and (iii) an own designed software, developed with Lab-Windows used to record and process the measurements. Validation was performed by analyzing spiked water samples containing pesticides. Biosensor for *in-situ* monitoring of organic phosphate pesticide as remote sensor was also developed for this purpose [102].

2.2.4. Reactivation of the Inhibited Enzyme

Inhibitions and re-activation characteristics of a biosensor by the organophosphate pesticides were investigated [103, 104]. Reactivation of an immobilized enzyme reactor was reported for the determination of acetylcholinesterase inhibitors in flow injection mode using 2-pyridinealdoxime methochloride [52, 96]. Pralidoxime iodide was also used as a reactivation agent for the inhibited AChE enzyme [53]. Table **2** summarized the most common enzymatic biosensor for indirect detection of pesticides.

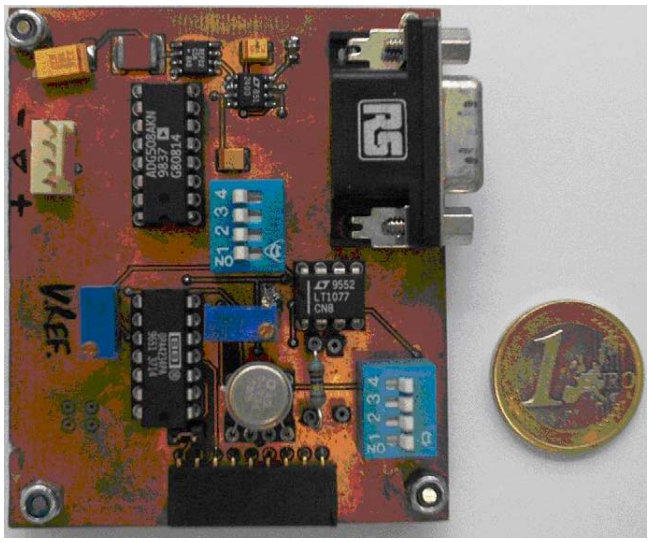

Fig. (12). Picture of the miniaturized electronic plate that functions as a potentiostat. (Talanta 75(2008)1208).

3. IMMUNOSENSORS

Immunosensors are based on the immunochemical reactions, i.e. binding of the antigen (*Ag*) to a specific antibody (*Ab*). Formation of such *Ab-Ag* complexes has to be detected under conditions where non-specific interactions are minimized. Each antigen (*Ag*) determination requires the production of a particular *Ab*, its isolation and, usually, its purification. In order to increase the sensitivity of immunosensors, enzyme labels are frequently coupled to *Ab* or *Ag*, thus requiring additional chemical synthesis steps. The enzyme activity being available only to quantify the amount of complex produced. The immunosensor consist of two processes,

Table 2. Enzymatic Biosensors for Indirect Detection of Pesticides

ANALYTE	SUBSTRATE	ENZYME	DETECTION LIMIT	REFS.
Trichlorfon	BuCh	BuChE	< 0.1 μM	[45]
Coumaphose/trichlorofon/aldicarb/methiocarb.	TCh	TChE	0.002/ 0.4/ 0.3 / 0.08 μg/ml	[46]
Carbaryl	ATCh	ATChE	5.0×10^{-5} mol/l	[47]
Carbafuran	ATCh	ATChE	at nM	[48]
Malthion	"	ATChE	0.03 ng/ml	[50]
Triazophose	''	ATChE	0.01μM	[53]
Paraoxon / chlorpyrifos ethyloxone	P-aminophenol	ChE	19.1/ 1.24 nM	[56]
chloropyrifosmethyl/ coumaphos/ carbofuran	TCh	TChE	2×10^{-8}, 5×10^{-8} and 8×10^{-8}M	[58]
Carbofuran / paraoxon	ACh / BuCh	AChE/BuChE	0.2 and 0.6nM	[64]
Chlorpyrifos ethyl oxon	TCh	ChE	1ppb	[65]
Dichlorvos/ Carbofuran	ACh/Ch	ChE/ChO	1.3×10^{-3} /0.01ppb	[76]
Carbaryl / Carbofuran	"	AChE/ChO	2 μg/ml	[69]
Trichlorofon	ACh/Ch/ H_2O_2	ChE/ChO/peroxidaze	5 ng/ml	[78]
Aldicarb	Glucose-6-phosphate/ glucose	AP/GOD	40 μg/ml	[80]
Methyl parathion/diazinon/Carbofuran/Carboy	Monophenol	Tyrosinase	6/19/5/10 ppb	[82]
Triazine	"	Tyrosinase	0.5×10^{-9} mol/l	[84]
Diazinon /dichlorvos	"	Tyrosinase	5 and 75 μM	[86]
Ziram/ diram/ zinc diethydithiocarbamate	"	Tyrosinase	0.074/1.3/1.7 μM	[87]
Zineb	Propinoaldehyde	Aldehyde dehydroganse	8 ppb	[92]
Herbicide	Pyruvate	Acetolactae synthase	1 μM	[93]
Ethyl paraoxon	Ascorbate	Ascorbate oxidase	1 ppm	[94]

ACh, acetylcholine ATCh, acetylthiocholine; BuCh, butyrylcholine BuTCh, butyrylthiocholine; TCh, thiocholine; Ch, choline; ATChE, Acetyl thiocholine estrase; BuTChE, butyryl thiocholine estrase; ChO, choline oxidaze; ChE, choline estrase; AP, acid phosphates; GOD, glucose oxidaze.

a molecular recognition process, for sensing the specific *Ag* - *Ab* binding reaction at the surface of receptor, and a signal-transfer process, for responding to changes in an electro-chemical parameter of the receptor caused by the specific binding. Important articles that focused on immunosensors for pesticide monitoring were described in the literatures [105-108].

3.1. Classification of Immunosensors

Depending on, whether labels are used or not, immunosensors are divided into two categories: labeled type and label-free type.

3.1.1. Labeled Formats

This procedure involves a label to quantify the amount of *Ab* or analyte bound during an incubation step. Widely used labels involve enzymes (e.g. glucose oxidase, horseradish peroxidase (HRP), β-galactosidase, alkaline phosphatase). Fig. (**13**) shows the schematic of labeled immunosensors. Commonly, two different formats for labeled immunosensors are available: sandwich assays and competitive assays. A sandwich assay consists of two recognition steps. In the first step, the *Ab* is immobilized on a transducer surface, allowing it to capture the analyte of interest. In the second step, labeled secondary *Ab* is added to bind with the previously captured analyte. The immunocomplexes (*immobilized Ab*-analyte-*labeled Ab*) are formed and the signals from labels increasing in proportion to the analyte concentration.

In competitive assays, the analyte competes with labeled analyte for a limited number of antibody binding sites. As the analyte concentration increase, more labeled analyte are displaced; giving a decrease in signal if antibody bound labeled analyte is detected.

3.1.2. Label - Free Formats

This procedure detects the binding of pesticide and the *Ab* on a transducer surface without any labels. There are also two basic types in this format: *direct* and *indirect*. In the first type, the response is directly proportional to the amount of pesticides present. The vital advantage of these direct immunosensors is a simple, single-stage reagentless operation. The second type, also based on competitive formats, is carried out as a binding inhibition test. The antigen (pesticide-

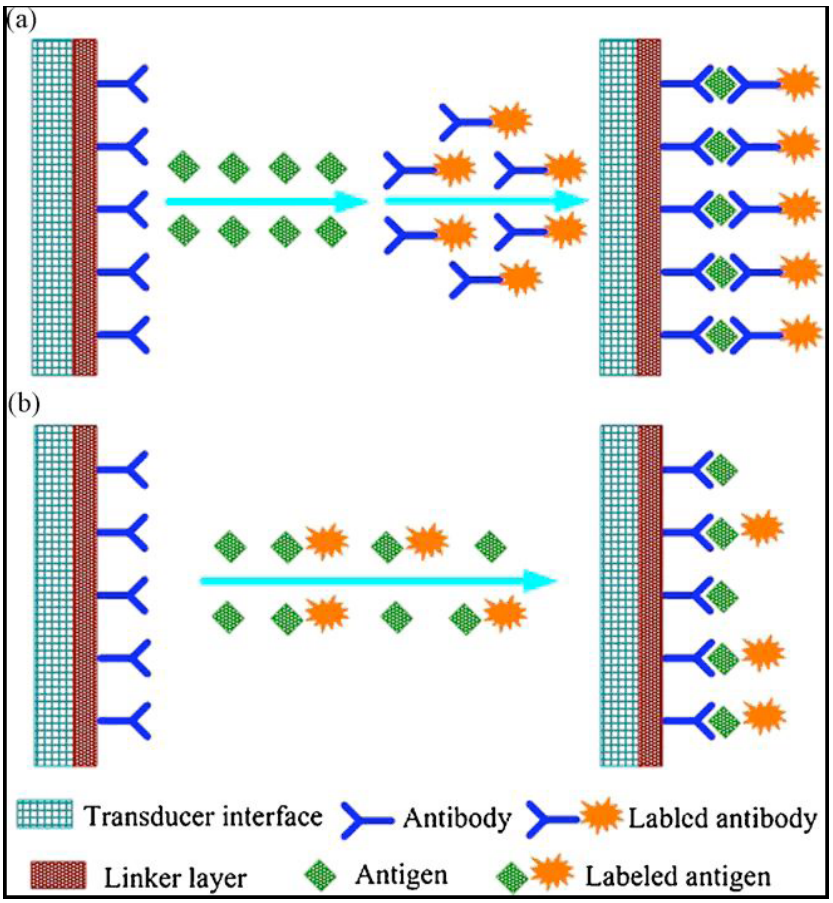

Fig. (13). Schematic of labeled immunosensors: (**a**) sandwich format and (**b**) competitive format. (Biosens. Bioelectronics 23(2008)1577).

protein conjugate) is first immobilized onto the surface of a transducer, and then pesticide-antibody mixtures are preincubated in solution. After being injected on the sensor surface, the antibody binding to the immobilized conjugate is inhibited by the presence of a target pesticide.

3.2. Electrochemical Immunosensors

The principle is based on the electrical properties of the electrode or buffer that is affected by *Ab-Ag* interaction. They can determine the level of pesticides by measuring the change of potential, current, conductance or impedance caused by the immunoreactions.

3.2.1. Potentiometric Methods

An immunosensor for the herbicide simazine was developed based on the potentiometric detection of peroxidase label after competitive immunoreaction on the electrode surface [109]. Gold planar electrodes were found to be the most effective supports for immunosensors. The activity of bound peroxidase was measured by basic pH shift of ascorbic acid solution after addition of hydrogen peroxide. The limit of simazine detection is 3 ng/ml. Another immunosensor for determination of simazine, based on ion selective field-effect transistor was also developed [110].

3.2.2. Amperometric Methods

An electrochemical immunosensor for the direct determination of paraoxon was developed based on the biocomposites of gold nanoparticles loaded with paraoxon antibodies [111]. The biocomposites are immobilized on the glassy carbon electrode (GCE) using Nafion membrane. Reduction and oxidation peaks located - 0.08 and - 0.03 mV versus SCE, respectively. The detection of paraoxon performed at - 0.03 mV is beneficial for sufficient selectivity. The immunosensor was employed for monitoring the concentrations of paraoxon in aqueous samples up to 1920 ng/ml with a detection limit of 12 ng/ml.

A separation-free bienzyme immunoassay system was developed for the electrochemical determination of the herbicide chlorsulfuron. Screen printed electrode with horseradish peroxidase as an integral component of the carbon ink was used as the detector [112]. A membrane with immobilized anti-chlorsulfuron antibodies was attached to the electrode. Free chlorsulfuron in the sample under test and a chlorsulfuron-glucose oxidase conjugate competed for the available binding sites of the membrane-immobilized antibodies. Addition of glucose, induced the generation of hydrogen peroxide by the glucose oxidase conjugate, which in turn was reduced by the peroxidase. The latter process caused an electrical current change, due to the direct re-reduction of peroxidase, which was measured to determine the chlorsulfuron content in the sample. The measuring range for chlorsulfuron detection was 0.01 - 1 ng/ml. The method was most suitable for *on-site* ecological monitoring.

Immunoassays for 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were carried out using a two-stage procedure involving (i) the isolation of the pesticides from the sample matrix by a specific

immunoreaction with immobilized antibodies and (ii) electrochemical detection of the unbound pesticide by its inhibition effect on the amperometric cholinesterase (*ChE*) biosensor [113]. Monoclonal *Ab* to 2, 4-D or polyclonal antisera to 2, 4,5-T were immobilized onto a nitrocellulose membrane. The ChE biosensor was immersed in the solution and the voltammogram was recorded by scanning the potential from - 0.1 to - 0.9 V at 1 V/s. The cathodic peak at - 0.55 V was used to calculate the pesticide concentration. The detection limits for 2,4-D and 2,4,5-T were 5 and 10pM, respectively. The method was applied to determine 2,4-D in milk following dilution to give a fat concentration of less than 1-1.5%.

A nitrocellulose film containing antibodies was immersed in the pesticide (2, 4-D) solution for 5 min. The film was transferred to an electrochemical cell, containing borate buffer. After separating the dissolved oxygen with a stream of H_2 the oscillopolarogram was recorded from - 0.1 to - 0.9 V *vs.* SCE and the height of the cathodic peak at - 0.55 V was measured [114]. The determination took ~25 min and the limit of detection was 10 pM.

3.2.3. Conductometry

Impedimetric immunosensor was developed for the determination of atrazine [115]. This method was described for the development of an electrochemical immunosensor, for the analysis of atrazine associated to biotinylated-Fab fragment K47 antibody. The sensors are based on mixed self-assembled monolayer consisting of 1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl)(biotinyl-PE) and 16-mercaptohexadecanoic acid. The properties of mixed monolayer were characterized by cyclic voltammetry and impedance spectroscopy. The electrical resistance, R_m decreases gradually after each building step of the sensing membrane. The results show that immunosensor based on this method is sensitive to atrazine antigen and a good linear response in the range 10 - 300 ng/ml. Table **3**, summarizes the different type of immunosensors used for detection of pesticides.

4. CELL-BASED BIOSENORS

Living micro-organisms (algae, bacteria, yeast and fungi) can be used as the biocatalytic elements for biosensors. Microbial (whole cells, pieces of cells) biosensors might be simpler and less expensive to develop for some applications, eliminating the need for isolation and purification of enzymes and related cofactors that are required for enzyme-based biosensors.

Amperometric microbial biosensor for direct determination of p-nitrophenyl-substituted organophosphate was developed. The biosensor comprised of p-nitrophenol degrader, *Pseudomonas putida JS444*, genetically engineered to express OPH on the cell surface immobilized on the carbon paste electrode [116]. The electrooxidization current of the intermediates was measured and correlated to the concentration of organophosphates. The detection limits were comparable to cholinesterase inhibition-based biosensors. Under optimum operating conditions the biosensor measured as low as 0.28, 0.26 and 0.29 ppb of paraoxon, methyl parathion, and parathion respectively.

A conductometric biosensor using immobilised *Chlorella vulgaris* microalgae as bioreceptors was used as a bi-enzymatic biosensor. Algae was immobilised inside bovine serum albumin membranes reticulated with glutaraldehyde vapours deposited on interdigitated conductometric electrodes [117]. Local conductivity variations caused by algae alkaline phosphatase and acetylcholinesterase activities could be detected. These organophosphorus pesticides for acetylcholinesterase, the bi-enzymatic biosensors were tested to study the influence of heavy metal ions and pesticides on the corresponding enzyme. For pesticides, initial experiments showed that paraoxon-methyl inhibits *Chlorella vulgaris* AChE contrary to parathion-methyl and carbofuran.

An amperometric microbial biosensor for the direct measurement of organophosphate nerve agents was described [118]. This sensor was based on the carbon paste electrode containing genetically engineered cells expressing OPH on the cell surface. Organophosphorus hydrolase catalyses the hydrolysis of organophosphorus pesticides with p-nitrophenyl substituent such as, paraoxon, parathion and parathion-methyl to p-nitrophenol. The later is detected anodically at the carbon transducer with the oxidation current being proportional to the nerve-agent concentration. The microbial biosensor had excellent storage stability, retaining 100 % of its original activity when stored at 4°C for a period of 45 days.

The biosensor was constructed by depositing a suspension of cultured *Escherichia coli* cells onto a polycarbonate membrane and mounting the membrane on a glass electrode by means of an O-ring [119]. The response of the biosensor for paraoxon, parathion, methyl parathion and diazinon was investigated. The effects on response of buffer concentration, pH and temperature were reported. Calibration graphs were not linear and the detection limits for all the analytes were 3μM.

Amicrobial biosensor consisting of a dissolved oxygen electrode modified with the genetically engineered PNP-degrader *Moraxella* sp. Displaying organophosphorus hydrolase on the cell surface for sensitive, selective, rapid and direct determination of p-nitrophenyl (PNP)-substituted organophosphates (OPs) was reported [120]. Operating at optimum conditions the biosensor was able to measure as low as 27.5 ppb of paraoxon and had excellent selectivity against triazines, carbamates and OPs without PNP substitutent.

5. DNA-BASED BIOSENSORS

DNA biosensors based on guanine oxidation have recently been proposed for detection of pesticides [121]. These DNA sensors utilize the interaction of DNA molecule with various compounds either by monitoring changes in the DNA redox properties (i.e. oxidation of guanine) or with an electro-active analyte intercalated on a DNA layer [122, 123]. Electrochemical techniques such as voltammetry [124-126], potentiometry [127] have been used to study the interaction of various compounds with DNA immobilized onto respective electrodes. A review article for electrochemical DNA biosensors was also published on this subject [128].

Double stranded *calf thymus* deoxyribonucleic acid entrapped polypyrrole-polyvinyl sulphonate (dsCT-DNA-PPy-PVS) films fabricated onto indium-tin-oxide (ITO) coated glass plates was used to detect organophosphates such as chlorpyrifos and malathion [129]. These biosensing elec-

Table 3. Immunosensors Detection of Pesticides

ANALYTE	IMMUNOSENSOR	SYSTEM	DETECTION LIMIT	REFS.
Simazine	Peroxidase label antibody	Potentiometry	3 ng/ml	[109]
Paraoxon	Paroxon antibodies	Amperometry	12 ng/ml	[111]
Chlorsulfuran	Anti-chlorsulfuron antibodies	"	0.01 ng/ml	[112]
2,4-D / 2,4,5-T	monocolonal/ polyclonal antibodies	"	5 / 10 PM	[113]
2,4-D	2,4-D	"	10 PM	[114]
Atrazine	Biotinylated-fabfragement K 47 antibody	conductommetry	10 ng/ml	[115]
Paraoxon / methyl parathion/ parathion	(Cell-based biosensor) microbial(*Pseudomonas putida JS444)*	Amperometry	0.28/ 0.26 /0.29 ppb	[116]
Paraoxon /parathion, methyl parathion /diazinon	cultured of *Escherichia coli* cells	Potentiometry	3μM	[119]
Paraoxon	Amicrobial (PNP- degrader *Moraxella*)	Amperometry	27.5 ppb	[120]
Chlorpyrifos /malathion	(DNA) *(Calf thymus-DNA)*	Amperometry	0.0016 / 0.17 ppm	[129]
Chlorpyrifos / malathion	Double stranded *calf thymus*-DNA	Voltammetry, FTIR, SEM, and electrochemical impedance	0.5 ppb and 0.01ppm	[130]

trodes have a response time of 30 s, they are stable for about 5 months when stored in desiccated conditions at 25 °C and can be used to amperometrically detect chlorpyrifos (0.0016 - 0.025 ppm) and malathion (0.17 to 5.0 ppm), respectively.

DNA biosensors are based on polyaniline (PANI)-polyvinyl sulphonate (PVS) and fabricated using electrochemical entrapment technique into indium-tin-oxide (ITO) for detection of organophosphorus pesticides (chlorpyrifos and malathion)[130]. These double stranded *calf thymus* bioelectrodes were characterized using square wave voltammetry, Fourier transform infra-red spectroscopy, scanning electron microscopy and electrochemical impedance techniques, respectively. These dsCT-DNA entrapped PANI-PVS/ITO bioelectrodes was found to have a response time of 30 s, with a stability of about 6 months and detection limit 0.5 ppb and 0.01ppm for chlorpyrifos and malathion, respectively.

6. FUTURE OUTLOOK

Biosensors play a successful role in environmental analysis and in process control. Examples include the analysis of pesticides and herbicides in aquatic samples. In environmental analysis, the advantage of immediate *on-site* analysis is of great advantage when attempting to ascertain the extent of pollution, for example, a lake. Laboratory based techniques required that samples be obtained over a wide area in order to delineate the area of contamination. *In situ* analysis would ensure that the extent of pollution would be known almost immediately, eliminating unnecessary sample analysis outside the polluted area as well as the cost of transporting samples back to the laboratory for analysis [100-102, 108, 112].

The use of a disposable biosensor offers some additional advantages such as mass production, possibility for miniaturization and low cost [65-69].

For monitoring purpose, biosensors should be regenerated after making a measurement. In enzyme-based biosensor, the use of some chemical reagents e.g. 2-pyridinealdoxime methochloride [52, 96] successfully regenerated the enzyme activity. The results clarified that proposed re-activation procedures could realize inexpensive and reliable continuous monitoring of organophosphate pesticides.

In case of immunosensor, two different strategies may be followed to achieve the renewal of the sensing surface:(1) breakage of the *Ab–Ag* bond and reusing the immunologic reagent immobilized in the solid phase; and (2) elimination of the *Ag-Ab* complex from the solid support and immobilization of fresh immunologic material [131]. In the first strategy, a careful selection of the dissociating agent must be made for efficiently dissociating the *Ag-Ab* complex without affecting association bonds between the support matrix and *Ab*. On the development of an immunosensor, for the organophosphorus pesticide ethyl parathion using ethyl parathion antibody, different dissociating agents were used [132]. The results reported in this investigation indicated that glycine-HCl (pH 2.3) buffer containing 1% dimethyl sulphoxide is a highly efficient dissociation buffer. In the second alternative, complete removal of the proteic material from the surface was achieved when using several regeneration solutions with extreme pH values and/or high salt concentrations [133].

Miniaturization is expected to have a marked impact on the development and applications of biosensensors. Miniaturization of a biosensor not only reduces the size of detection device and sample volume, but also integrates all steps of the analytical process into a single-sensor device. Thus, it results in reduction of both the time and cost of analysis. Moreover, it is expected to lead to a further portability for *in vivo* sensing and *in-field* applications. The miniaturization trend involves adaptation of microfabrication and nanofabri-

cation techniques, such as microelectrode systems and combined with microelectronic circuitry [100] sometimes referred to as "smart sensor systems", which in turn can be configured into highly portable [101-102] and inexpensive handheld instrumentation.

Since a number of pesticides have a similar mode of action affecting the activity of the same enzyme, most of enzyme-based biosensors are used for screening purpose and are unspecific for individual pesticides. They can only detect total pesticides content and do not provide specific information about a particular pesticide.

Immunosensors are biosensors that use *Ab* or *Ag* as the specific sensing element and provide concentration-dependent signals. They appear to be appropriate for identification of a single pesticide or in some cases, small groups of similar pesticides in environmental monitoring, as they are rapid, specific and cost-effective analytical devices [134].

Since new developments in protein engineering can contribute to the improvement of a novel Ab, antibody fragment represent the next generation of immunochemical reagents extending options of poly- and mono-colonal Ab for application in to pesticide, environmental and food analysis [115-135].

REFFERNCES

[1] Arduini, F.; Ricci, F.; Tuta, C.S.; Moscone, D.; Amine, A.; Palleschi, G. Detection of carbamic and organophosphours pesticides in water samples using a cholinesterase biosensor based on Prussian blue-modified screen-printed electrode. *Anal. Chim. Acta*, **2006**, *580*, 155-162.

[2] Li, B.X.; He, Y.Z.; Xu, C.L. Simultaneous determination of three organ phosphorus pesticides residues in vegetables using continuous-flow chemiluminescence with artificial neural network calibration. *Talanta*, **2007**, *72*, 223-30.

[3] FAO, Agriculture towards. In: *Proceedings of the C 93/94 Document of 27th Session of the FAO Conference*, Rome, **1993**.

[4] Aspelin, L. *Pesticides Industry Sales and Usage*, **1992** and 1993 Market Estimates, US Environmental Protection Agency: Washington, **1994**.

[5] U.S. FDA. Pesticide monitoring databases; Available from: http:// www.cfsan.fda.fda.gov/~lrd/pestadd.html.

[6] Dikshith, T.S.S. Pesticides. In: Dikshith, T.S.S. Ed.,"*Toxicology of Pesticides in Animals*". CRC Press: Boston **1991**; pp. 1-39.

[7] Chapalamadugu, S.; Chaudhry, G.S. Microbiological and biotechnological aspects of metabolism of carbamates and organophosphates. *Crit. Rev. Biotechnol*., **1992**, *12*, 357-89.

[8] American Public Health Association Ed., Standard Methods for Examination of Water and Wastewater, 20th ed., American Public Health Association: Washington, **1998**, pp. 6/85–6/90.

[9] EPA Method 8141 A, US Environmental Protection Agency, **2000**.

[10] Vicente, A.; Yolanda, P. Determination of pesticides and their degradation products in soil: critical review and comparison of methods. *Trends Anal. Chem.*, **2004**, *23*, 772-89.

[11] Ferrer, I.; Garcia-Reyes, J.F.; Fernandez-Alba, A. Identification and quantitation of pesticides in vegetables by liquid chromatography time-of-flight mass spectrometry. *Trends. Anal. Chem.*, **2005**, *24*, 671-82.

[12] Hernandez, F.; Sancho, J.V.; Pozo, O.J. Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. *Anal. Bioanal. Chem.*, **2005**, *382*, 934-46.

[13] Donald, G.B. *Biosensors, theory and applications*. Published in the Western Hemisphere: USA. **1993**.

[14] Guilbault, G.G.; Pravda, M.; Kreuzer, M. Biosensors-42 years and counting. *Anal. Lett*., **2004**, *37*, 14481-96.

[15] Gopel, W.; Jones, T.A.; Kleitz, M.; Lundstrom, I.; Sieyama, T. Chemical and biochemical sensors. In Sensors, *A comprehensive survey*. Gopel, W.; Hess, H.; Zemel, J.N.; Eds. CRC. Publlisher: New York, **1991**, vols. 2 & 3.

[16] Bartic, C.; Borghs, G. Organic thin-film transistors as transducers for (bio) analytical applications. *Anal. Bioanal. Chem.*, **2006**, *384*, 354-65.

[17] Dzyadevych, S.V.; Arkhypova, V.N.; Martelet C.; Jaffrezic-Renault N.; Chovelon, J.M.; El'skaya, A.V.; Soldatkin A.P. Potentiometric biosensors based on ISFETs and immobilized cholinesterases. *Electroanalysis*, **2004**, *16*, 1873-82.

[18] Dzyadevych, S.V.; Soldatkin, A.P.; Korpan, Y.I.; Arkhypova, V.N.; El'skaya, A.V.; Chovelon, J.M.; Martelet, C.; Jaffrezic-Renault, N. Biosensors based on enzyme field-effect transistors for determination of some substrates and inhibitors. *Anal. Bioanal. Chem.*, **2003**, *377*, 496-506.

[19] Tuan, M.A.; Sergei, V.D.; Minh, C.V.; Nicole, J.R., Chien, N. D.; Jean-Marc C. Conductometric tyrosinase biosensor for the detection of diuron, atrazine and its main metabolites. *Talanta*, **2004**, *63*, 365-70.

[20] Cullen, D.C.; Sethi, R.S.; Lowe, C.R. Multi-analyte miniature conductance biosensor. *Anal. Chim. Acta*, **1990**, *231*, 33-40.

[21] Thevenot, D.R.; Toth, K.; Durst, R.A.; Wilson, G.S. Electrochemical biosensors: recommended definitions and classification. *Biosens. Bioelectron*, **2001**, *16*, 121-31.

[22] Andreescu, S.; Sadik, O.A. Trends & challenges in biochemical sensors for clinical and environmental monitoring. *Pure Appl. Chem*., **2004**, *76*, 861-78.

[23] Daniel, R.T.; Klara, T.; Richard, A.D.; George S.W. Electrochemical biosensors: recommendation definitions and classification, IUPAC. *Pure Appl. Chem*., **1999**, *71*, 2333-48.

[24] Bilitewski, U.; Turner, A.P.F. *Bioseneors for Environmental Monitoring*. Harwood Academic Publishers: UK, **2000**.

[25] Hanane, Z.; José, L. H. H.; de C. I. N.-R.; Baohong, L.; Khalid, R. T.; Jean-Louis, M. Alumina sol–gel/sonogel-carbon electrode based on acetylcholinesterase for detection of organophosphorus pesticides. *Talanta*, **2008**, *77*, 217-21.

[26] Dan, D.; Jiawang, D.; Jie, C.; Jianming, Z.; Li, L. *In situ* electrodeposited nanoparticles for facilitating electron transfer across self-assembled monolayers in biosensor design. *Talanta*, **2008**, *74*, 1337-43.

[27] Anitha, K.; Mohan, S.V.; Reddy, S.J. Development of acetylcholinesterase silica sol-gel immobilized biosensor - an application towards oxydemeton methyl detection. *Biosens. Bioelectron*., **2004**, *20*, 848-56.

[28] Carr, P.W.; Bowers, L.D. Theory and applications of enzyme electrodes. In: *Immobilized Enzymes in Analytical and Clinical Chemistry: Fundamentals and Applications*. John Wiley & Sons, New York, **1980**, pp. 197-310.

[29] Andreescu, S.; Bucur, B.; Marty, J. L. Affinity immobilization of tagged enzymes. In: Guisan, J.M. Ed., *Immobilization of Enzymes and Cells*, 2nd ed. Humana Press: USA, **2006**.

[30] Bucur, B.; Danet, A.F.; Marty, J. L. Cholinesterase immobilisation on the surface of screen-printed electrodes based on concanavalin affinity. *Anal. Chim. Acta*, **2005**, *530*, 1-6.

[31] Ashok, M.; Wilfred, C.; Priti, M.; Joseph, W.; Kim, R. R. Biosensors for direct determination of organophosphate pesticides. *Biosens. Bioelectron*, **2001**, *16*, 225-30.

[32] Amine, A.; Mohammadi, H.; Bouais, I.; Palleschi, G. Enzyme inhibition-based biosensors for food safety and environmental monitoring. *Biosens. Bioelectron*, **2006**, *21*, 1405-23.

[33] Simonian, A. L.; Flounders, A.W.; Wild, J.R.; FET-based biosensors for the direct detection of organophosphate neurotoxins. *Electroanalysis*, **2004**, *16*, 1896-1906.

[34] Schoening, M.J.; Arzdorf, M.; Mulchandani, P.; Chen, W.; Mulchandani, A. Towards a capacitive enzyme sensor for direct determination of organophosphorus pesticides: fundamental studies and aspects of development. *Sensors*, **2003**, *3*, 119-27.

[35] Simonian, A.L.; Grimsley, J.K.; Flounders, A.W.; Schoeniger, J.S.; Cheng, T.C.; DeFrank, J.J.; Wild, J.R. Enzyme-based biosensor for the direct detection of fluorine-containing organophosphates. *Anal. Chim. Acta*, **2001**, *442*, 15-23.

[36] Sacks, V.; Eshkenazi, I.; Neufeld, T.; Dosoretz, C.; Rishpon, J. Immobilized parathion hydrolase: an amperometric sensor for parathion. *Anal. Chem.*, **2000**, *72*, 2055-58.

[37] Chough, S.H.; Mulchandani, A.; Mulchandani, P.; Chen, W.; Wang, J.; Rogers, K.R. Organophosphorus hydrolase-based amperometric sensor: modulation of sensitivity and substrate selectivity. *Electroanalysis*, **2002**, *14*, 273-76.

[38] Mulchandani, P., Chen, W.; Mulchandani, A. Flow-injection amperometric enzyme biosensor for direct determination of organophosphate nerve agents. *Environ. Sci. Technol.*, **2001**, *35*, 2562-65.

[39] Schoening, M.J.; Krause, R.; Block, K.; Musahmen, M.; Mulchandani, A.; Wang, J. A dual amperometric/potentiometric FIA-based biosensor for the distinctive detection of organophosphorus pesticides. *Sens. Actuators B*, **2003**, *95*, 291-96.

[40] Wang, J.; Krause, R.; Block, K.; Musameh, M.; Mulchandani, A.; Mulchandani, P.; Chen, W.; Schoening, M.J. Dual amperometric-potentiometric biosensor detection system for monitoring organophosphorus neurotoxins. *Anal. Chim. Acta*., **2002**, *465*, 197-203.

[41] Trojanowicz, M. Determination of pesticides using electrochemical enzymatic biosensors. *Electroanalysis*, **2002**, *14*, 1311-28.

[42] Nunes, G.S.; Barcelo, D. Electrochemical biosensors for pesticide determination in food samples. *Analysis*, **1998**, *26*, M156-M59.

[43] Silvia, S.; Arben, M. Determination of Toxic substances based on enzyme inhibition. Part1.Electrochemical biosensors for the determination of pesticides using batch procedures. *Crit. Rev. Anal. Chem.*, **2003**, *33*, 89-126.

[44] Silvana, A.; Jean-Louis, M. Twenty years in cholinesterase biosensors: From basic research to practical applications. *Biomol. Eng.*, **2006**, *23*, 1-15.

[45] Reybier, K.; Zairi, S.; Jaffrezic-Renault, N. The use of polyethylenimine for fabrication of potentiometric cholinesterase biosensors. *Talanta*, **2002**, *56*, 1015-20.

[46] Ivanov, A.N.; Lukachova, L.V.; Evtugyn, G.A.; Karyakina, E.E.; Kiseleva, S.G.; Budnikov, H.C.; Orlov, A.V.; Karpacheva, G.P.; Karyakin, A.A. Polyaniline-modified cholinesterase sensor for pesticide determination. *Bioelectrochemistry*, **2002**, *55*, 75-78.

[47] Josiane, C.; Sergio, A.S. M. Determination of carbaryl in tomato "in natura" using an amperometric biosensor based on the inhibition of acetylcholinesterase activity. *Sens. Actuators B*, **2008**, *129*, 40-46.

[48] Olga, S.; Jon, R. K. An acetylcholinesterase enzyme electrode stabilized by an electrodeposited gold nanoparticle layer. *Electrochem. Commun.*, **2007**, *9*, 935-40.

[49] Dan, D.; Shizhen, C.; Jie, C.; Aidong, Z. Electrochemical pesticide sensitivity test using acetylcholinesterase biosensor based on colloidal gold nanoparticle modified sol–gel interface. *Talanta*, **2008**, *74*, 766-72.

[50] Dan, D.; Jiawang, D.; Yuan, T.; Xi, C. Application of chemisorption/desorption process of thiocholine for pesticide detection based on acetylcholinesterase biosensor. *Sens. Actuators B*, **2008**, *134*, 908-12.

[51] Moore, R.R.; Banks, C.E.; Compton, R.G. Electrocatalytic detection of thiols using an edge plane pyrolytic graphite electrode. *Analyst*, **2004**, *129*, 755-58.

[52] Shu, P. Z.; Lian, G.S.; Zhen, R. T.; Yi, Z.; Li, Y. S.; Deng, S. Z. Study of enzyme biosensor based on carbon nanotubes modified electrode for detection of pesticides residue. *Chinese Chem. Lett.*, **2008**, *19*, 592-594.

[53] Dan, D.; Xi, H.; Jie, C.; Aidong, Z. Amperometric detection of triazophos pesticide using acetylcholinesterase biosensor based on multiwall carbon nanotube-chitosan matrix. *Sens. Actuators B*, **2007**, *127*, 531-35.

[54] Bonnet, C.; Andreescu, S.; Marty, J.L. Adsorption: an easy and efficient immobilization of acetylcholinesterase on screen-printed electrodes. *Anal. Chim. Acta*, **2003**, *481*, 209-11.

[55] Gogol, E.V.; Evtugyn, G.A.; Marty, J.L.; Budnikov, H.C.; Winter, V.G. Amperometric biosensors based on Nafion-coated screen-printed electrodes for the determination of cholinesterase inhibitors. *Talanta*, **2000**, *53*, 379-89.

[56] Andreescu, S.; Noguer, T.; Magearu, V.; Marty, J.L. Screen-printed electrode based on acetylcholinesterase for the detection of pesticides in presence of organic solvents. *Talanta*, **2002**, *22*, 169-76.

[57] Silva-Nunes, G.; Jeanty, G.; Marty, J.L. Enzyme immobilization procedures on screen-printed electrodes used for the detection of anticholinesterase pesticides. Comparative study. *Anal. Chim. Acta*, **2004**, *523*, 107-15.

[58] Ivanov, A.; Evtugyn, G.; Budnikov, H.; Ricci, F.; Moscone, D.; Palleschi, G. Cholinesterase sensors based on screen-printed electrodes for detection of organophosphorus and carbamic pesticides. *Anal. Bioanal. Chem.*, **2003**, *377*, 624-31.

[59] Nikolay, A. P.; Paul, A. M. A novel procedure for rapid surface functionalisation and mediator loading of screen-printed carbon electrodes. *Anal. Chim. Acta*, **2008**, *612*, 190-97.

[60] Serena, L.; Dominika, O.; Ilaria, P.; Marco, M. Evaluation of pesticide-induced acetylcholinesterase inhibition by means of disposable carbon-modified electrochemical biosensors. *Enzyme Microb. Technol.*, **2007**, *40*, 485-89.

[61] Crew, A.; Hart, J.P.; Wedge, R.; Marty, J. L.; Fournier, D. A screen-printed, amperometric, biosensor array for the detection of organophosphate pesticides based on inhibition of wild type, and mutant acetylcholinesterases, from Drosophila melanogaster. *Anal. Lett.*, **2004**, *37*, 1601-10.

[62] Ricci, F.; Palleschi, G. Sensor and biosensor preparation, optimization and applications of Prussin Blue modified electrode. *Biosens. Bioelectron*, **2005**, *21*, 389-407.

[63] Fabiana, A.; Francesco, R.; Catalin, S. T.; Danila, M.; Aziz, A.; Giuseppe, P. Detection of carbamic and organophosphorous pesticides in water samples using a cholinesterase biosensor based on Prussian Blue-modified screen-printed electrode *Anal. Chim. Acta*, **2006**, *580*, 155-62.

[64] Albareda-Sirvent, M.; Merkoci, A.; Alegret, S. Pesticide determination in tap water and juice samples using disposable amperometric biosensors made using thick-film technology. *Anal. Chim. Acta*, **2001**, *31*, 35-44.

[65] Montesinos, T.; Perez-Munguia, S.; Valdez, F.; Marty, J.L. Disposable cholinesterase biosensor for the detection of pesticides in water-miscible organic solvents. *Anal. Chim. Acta*, **2001**, *431*, 231-37.

[66] Bachmann, T.T.; Leca, B.; Villatte, F.; Marty, J.-L.; Fournier, D.; Schmid, R.D. Improved multianalyte detection of organophosphate and carbamate with disposable multielectrode biosensors using recombinant mutants of Drosophila Acetylcholinesterase and artificial neutral network. *Biosens. Bioelectron*, **2000**, *15*, 193-201.

[67] Joshi, K.A.; Tang, J.; Haddon, R.; Wang, J.; Chen, W.; Mulchaldani, A. A disposable biosensors for organophosphorus nerve agents based on carbon nanotubes modified thick film strip electrodes. *Electroanalysis*, **2005**, *17*, 54-58.

[68] Bachmann, T.T.; Schmidt, R.D.; A disposable multielectrode biosensor for rapid simultaneous detection of the insecticides paraoxon and carbamate at high resolution. *Anal. Chim. Acta*, **1999**, *401*, 95–103.

[69] Palchetti, I.; Cagnini, A.; Del Carlo, M.; Coppi, C.; Mascini, M.; Turner, A.P.F. Determination of acetylcholinesterase pesticides in real samples using a disposable biosensor. *Anal. Chim. Acta*, **1997**, *337*, 315-321.

[70] Graziella, L.T.; Marinella, S.T. Synergetic effect of organic solvents and paraoxon on the immobilized acetylcholinesterase. *Pes. Biochem. Physiol.*, **2008**, *90*, 73-81.

[71] Gabriela, V.; Didier, F.; Maria, T. R.; Sensitive amperometric biosensor for dichlorovos quantification: application to detection of residues on apple skin. *Talanta*, **2008**, *74*, 741-46.

[72] Mionetto, N.; Marty, J.L.; Karube, I. Acetylcholinesterase in organic solvents for the detection of pesticides: biosensor application. *Biosens. Bioelectron*, **1994**, *9*, 463-470.

[73] Andreescu, S.; Noguer, T.; Magearu, V.; Marty, J. L. Screen-printed electrode based on AChE for the detection of pesticides in presence of organic solvents. *Talanta*, **2002**, *57*, 169-76.

[74] Trojanowicz, M.; Hitchman, M.L.; Determination of pesticides using electrochemical biosensors. *Trends. Anal. Chem.*, **1996**, *15*, 38-45.

[75] Lin, Y.H.; Lu., F.; Wang, J.; Disposable carbon nanotube-modified screen-printed biosensor for amperometric detection of organophosphorus pesticides and nerve agents. *Electroanalysis*, **2004**, *16*, 145-149.

[76] Snejdarkova, M.; Svobodova, L.; Nikolelis, D.P.; Wang, J.; Hianik, T. Acetylcholine biosensor based on dendrimer layers for pesticides detection. *Electroanalysis*, **2003**, *15*, 1185-1191.

[77] Kok, F.N.; Hasirci, V. Determination of binary pesticide mixtures by an acetylcholinesterase-choline oxidase biosensor. *Biosens. Bioelectron.*, **2004**, *19*, 661-65.

[78] Mazei, F.; Botre, F.; Botre, C. Acid phosphatase/glucose oxidase-based biosensors for the determination of pesticides. *Anal. Chim. Acta*, **1996**, *336*, 67-75.

[79] Diehl-Faxon, J.; Ghindilis, A.L.; Atanasov, P.; Wilkins, E. Direct electron-transfer-based trienzyme electrode for monitoring of organophosphorus pesticides. *Sens. Actuators B*, **1996**, *36*, 448-57.

[80] Mazzei, F.; Botre, F.; Botre, C. Acid phosphatase/glucose oxidase-based biosensors for the determination of pesticides. *Anal. Chim. Acta*, **1996**, *336*, 67-75.

[81] Danzer, T.; Schwedt, G. Chemometric methods for the development of a biosensor system and the evaluation of inhibition studies with solutions and mixtures of pesticides and heavy metals: Part 1. Development of an enzyme electrodes system for pesticide and heavy metal. *Anal. Chim. Acta*, **1996**, *318*, 275-286.

[82] Yaico, D., Tanimoto de, A.; Lucas, F.F. Amperometric biosensing of carbamate and organophosphate pesticides utilizing screen-printed tyrosinase-modified electrodes. *Anal. Chim. Acta*, **2007**, *596*, 210-21.

[83] Letter to the Editor. Substrate-bound tyrosinase electrode using gold nanoparticles anchored to pyrroloquinoline quinone for a pesticide biosensor. *Sens. Actuators B*, **2008**, *133*, 1-4.

[84] Campanella, L.; Dragone, R.; Lelo, D.; Martini, E.; Tomassetti, M. Tyrosinase inhibition organic phase biosensor for triazinic and benzotriazinic pesticide analysis (part two). *Anal. Bioanal. Chem.*, **2006**, *384*, 915-21.

[85] Vidal, J.C.; Esteban, S.; Gil, J. A comparative study of immobilization methods of a tyrosinase enzyme on electrodes and their application to the detection of dichlorvos organophosphorus insecticide. *Talanta*, **2006**, *68*, 791-99.

[86] Everett, W.R.; Rechnitz, G.A. Mediated bioelectrocatalytic determination of organophosphorus pesticides with a tyrosinase-based oxygen biosensor. *Anal. Chem.*, **1998**, *70*, 807-810.

[87] Perez-Pita, M.T.; Reviejo, A.J.; Manuel-de-Villena, F.J.; Pingarron, J.M. Amperometric selective biosensing of dimethyl- and diethyldithiocarbamates based on inhibition processes in a medium of reversed micelles. *Anal. Chim. Acta*, **1997**, *340*, 89-97.

[88] Everett, W.R.; Rechnitz, G.A. Enzyme-based electrochemical biosensors for determination of organophosphorus and carbamate pesticides. *Anal. Lett.*, **1999**, *32*, 1-10.

[89] Campanella, L.; Bonanni, A.; Martini, E.; Todini, N.; Tomassetti, M. Determination of triazine pesticides using a new enzyme inhibition tyrosinase OPEE operating in chloroform. *Sens. Actuators B*, **2005**, *111-112*, 505-14.

[90] Martly, J.L.; Mionetto, N.; Nogure, T.; Ortega, F.; Roux, C. Enzyme sensors for the detection of pesticides. *Biosens. Bioelectron.*, **1993**, *8*, 273-80.

[91] Noguer, T.; Marty, J.L. High sensitive bienzymic sensor for the detection of dithiocarbamate fungicides. *Anal. Chim. Acta*, **1997**, *347*, 63-70.

[92] Noguer, T.; Gradinaru, A.; Ciucu, A.; Marty, J.L. A new disposable biosensor for the accurate and sensitive detection of ethylenebis (dithiocarbamate) fungicides. *Anal. Lett.*, **1999**, *32*, 1723-38.

[93] Seki, A., Ortega, F.; Marty, J.L. Enzyme sensor for the detection of herbicides inhibiting acetolactate synthase. *Anal. Lett.*, **1996**, *29*, 1259-71.

[94] Rekha, K.; Gouda, M.D.; Thakur, M.S.; Karanth, N.G. Ascorbate oxidase based amperometric biosensor for organophosphorus pesticide monitoring. *Biosens. Bioelectron.*, **2000**, *15*, 499-502.

[95] Shi, M.H.; Xu, J.J.; Zhang S.; Liu, B.H.; Kong, J.L. A mediator-free screen-printed amperometric biosensor for screening of organophosphorus pesticides with flow-injection analysis (FIA) system. *Talanta*, **2006**, *68*, 1089-1095.

[96] Meng, F.P.; He, D.H.; Zhu, X.S.; Yang, Z.X.; Ma, D.D. Detection of malathion in seawater using a flow injection type biosensor based on the immobilized acetylcholinesterase. *Fenxi. Huaxue*, **2005**, *33*, 922-26.

[97] Jeanty, G.; Wojciechowska, A.; Marty, J. L.; Trojanowicz, M. Flow-injection amperometric determination of pesticides on the basis of their inhibition of immobilized acetylcholinesterases of different origin. *Anal. Bioanal. Chem.*, **2002**, *373*, 691-95.

[98] Valdés-Ramírez, G.; Gutiérrez, M.; del Valle, M.T.; Ramírez-Silva, M. T.; Fournier, D.; Marty, J.L. Automated resolution of dichlorvos and methylparaoxon pesticide mixtures employing a Flow Injection system with an inhibition electronic tongue. *Biosens. Bioelectron*, **2009**, *24*,1103-08.

[99] Bachmann, T.T.; Leca, B.; Vilatte, F.; Marty J.L., Fournier, D.; Schmid, R.D. Improved multianalyte detection of organophosphates and carbamates with disposable multielectrode biosensors using recombinant mutants of Drosophila acetylcholinesterase and artificial neural networks. *Biosens. Bioelectron*, **2000**, *15*, 193-201.

[100] Hildebrandt, A.; Bragó´ s, R.; Lacorte, S.; Marty, J.L. Performance of a portable biosensor for the analysis of organophosphorus and carbamate insecticides in water and food. *Sens. Actuators B*, **2008**, *133*, 195-201.

[101] Alain, H.; Jordi, R.; Ramon, B.; M`arius, T.; S´ılvia, L. Development of a portable biosensor for screening neurotoxic agents in water samples. *Talanta*, **2008**, *75*, 1208-13.

[102] Wang, J.; Chen, L.; Mulchandani, A.; Mulchandani, P.; Chen, W. Remote biosensor for *in-situ* monitoring of organophosphate nerve agents. *Electroanalysis*, **1999**, *11*, 866-69.

[103] Okazaki, S.; Nakagawa, H.; Fukuda, K.; Asakura, S.; Kiuchi, H.; Shigemori T.; Takahashi, S. Re-activation of an amperometric organophosphate pesticide biosensor by 2-pyridinealdoxime methochloride. *Sens. Actuators B*, **2000**, *66*, 131-34.

[104] Garcia de Maria, C.; Munoz, T.M.; Townhend, A. Reactivation of an immobilized enzyme reactor for the determination of acetylcholinesterase inhibitors. Flow injection determination of paraoxon. *Anal. Chim. Acta*, **1994**, *295*, 287-96.

[105] Xuesong, J.; Dongyang, L.; Xia, X.; Yibin, Y.; Yanbin, Li ; Zunzhong, Y. Immunosensors for detection of pesticide residues. *Biosens. Bioelectron*, **2008**, *23*, 1577-87.

[106] Ciumasu, I.M.; Kramer, P.M.; Weber, C.M.; Kolb, G.; Tiemann, D.; Windisch, S.; Frese, I.; Kettrup, A.A. A new versatile field immunosensor for environmental pollutants. Development and proof of principle with TNT, diuron, and atrazine. *Biosens. Bioelectron*, **2005**, *21*, 354-64.

[107] Zacco, E.; Galve, R.; Marcob, M.P.; Alegret, S., Pividori, M.I. Electrochemical biosensing of pesticide residues based on affinity biocomposite platforms. *Biosens. Bioelectron*, **2007**, *22*, 1707-15.

[108] Tschmelak, J.; Proll, G.; Riedt, J.; Kaiser, J.; Kraemmer, P.; Barzaga, L.; Wilkinson, J.S.; Hua, P.; Hole, J.P.; Nudd, R.; Jackson, M.; Abuknesha, R.; Barcelo, D.; Rodriguez-Mozaz, S.; Lopez de Alda, M.J.; Sacher, F.; Stien, J.; Slobodnik, J.; Oswald, P.; Kozmenko, H.; Korenkova, E.; Tothova, L.; Krascsenits, Z.; Gauglitz, G. Automated Water Analyser Computer Supported System (AWACSS) Part II: Intelligent, remote-controlled, cost-effective, on-line, water-monitoring measurement system. *Biosens. Bioelectron*, **2005**, *20*, 1509-19.

[109] Yulaev, M.F.; Sitdikov, R.A.; Dmitrieva, N.M.; Yazynina, E.V.; Zherdev, A.V.; Dzantiev, B.B. Development of a potentiometric immunosensor for herbicide simazine and its application for food testing. *Sens. Actuators. B*, **2001**, *75*, 129-35.

[110] Starodub, N.F.; Dzantiev, B.B. ; Starodub, V.M.; Zherdev, A.V. Immunosensor for the determination of the herbicide simazine based on an ion-selective field-effect transistor. *Anal. Chim. Acta*, **2000**, *424*, 37-43.

[111] Hu, S.Q.; Xie, J.W.; Xu, Q.H.; Rong, K.T.; Shen, G. L.; Yu, R.Q. A label-free electrochemical immunosensor based on gold nanoparticles for detection of paraoxon. *Talanta*, **2003**, *61*, 769-77.

[112] Dzantiev, B.B.; Yazynina, E.V.; Zherdev, A.V.; Plekhanova, Y.V.; Reshetilov, A.N.; Chang, S.C.; McNeil, C.J. Determination of the herbicide chlorsulfuron by amperometric sensor based on separation-free bienzyme immunoassay. *Sens. Actuators B*, **2004**, *98*, 254-61.

[113] Medyantseva, E.P.; Vertlib, M.G.; Kutyreva, M.P.; Khaldeeva, E.I.; Budnikov, G.K.; Eremin, S.A. The specific immunochemical detection of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid pesticides by amperometric cholinesterase biosensors. *Anal. Chim. Acta*, **1997**, *347*, 71-78.

[114] Medyantseva, E.P.; Vertlib, M.G.; Budnikov, G.K.; Babkina, S.S.; Eremin, S.A. Amperometric biochemical sensor based on immobilized cholinesterase in the immunoassay of pesticides. *Zh. Anal. Khim.*, **1995**, *50*, 782-786.

[115] Hleli, S.; Martelet, C.; Abdelghani, A. Atrazine analysis using an impedimetric immunosensor based on mixed biotinylated self-assembled monolayer. *Sens. Actuators B*, **2006**, *113*, 711-17.

[116] Lei, Y., Mulchandani, P., Wang, J., Chen, W., Chen, W.; Mulchandani, A. Highly sensitive and selective amperometric microbial biosensor for direct determination of p-nitrophenyl-substituted organophosphate nerve agents. *Environ. Sci. Technol.*, **2005**, *39*, 8853-57.

[117] Chouteau, C.; Dzyadevych, S.; Durrieu, C.; Chovelon, J. M. A bienzymatic whole cell conductometric biosensor for biosensor for heavy metal ions and pesticides detection in water samples. *Biosens. Bioelectron*, **2005**, *21*, 273-81.

[118] Mulchandani, P.; Chen, W.; Mulchandani, A.; Wang, J.; Chen, L. Amperometric microbial biosensor for direct determination of or-

ganophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. *Biosens. Bioelectron*, **2001**, *16*, 433-37.

[119] Mulchandani, A.; Mulchandani, P.; Chauhan, S.; Kaneva, I.; Chen, W. A potentiometric microbial biosensor for direct determination of organophosphate nerve agents. *Electroanalysis*, **1998**, *10*, 733-37.

[120] Priti, M.; Wilfred, C.; Ashok, M. Microbial biosensor for direct determination of nitrophenyl-substituted organophosphate nerve agents using genetically *Moraxella* sp. *Anal. Chim. Acta*, **2006**, *568*, 217-221.

[121] Wang, J.; Rivas, G.; Cai, E.; Palecek, P.; Nielsen, H; Shiraishi, N.; Dontha, D., Luo, C.; Parrado, M.; Chicarro, P.A.M.; Farias, F.S.; Valera, D.H.; Grant, M.; Ozsoz, M.; Flair, M.N. DNA electrochemical biosensors for environmental monitoring. A Review. *Anal. Chim. Acta*, **1997**, *347*, 1-8.

[122] Wang, J. Electrochemical nucleic acid biosensors. *Anal. Chim. Acta*, **2002**, *569*, 63-71.

[123] Mascini, M.; Palchetti I.; Marrazza, G. DNA electrochemical biosensors. *Fr. J. Anal. Chem.*, **2001**, *369*, 15-22.

[124] Arora, K.; Chaubey, A.; Singhal. R.; Singh, R.P.; Samanta, S.B.; Chand, S.; Malhotra, B.D. Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films to DNA biosensors. *Biosens. Bioelectron.*, **2006**, *21*, 1777-83.

[125] Lucrelli, F.; Kicela, A.; Palchetti, G.; Marrazza, G.; Mascini, M. Electrochemical DNA biosensor for analysis of wastewater samples. *Bioelectrochemistry*, **2002**, *58*, 113-118.

[126] Erdem, A.; Kerman, K.; Meric, B.; Akarca, U.S.; Ozsoz, M., Novel hybridization indicator methylene blue for the electrochemical detection of short DNA sequences related to the hepatitis B virus. *Anal. Chim. Acta*, **2000**, *422*, 139-49.

[127] Chitti, G.; Marrazza, G.; Mascini, M. Toxicity assessment of organic pollution in wastewater using a bacterial biosensor. *Anal. Chim. Acta*, **2001**, *47*, 155-165.

[128] He, P.G.; Xu, Y.; Fang, Y.Z. A review: electrochemical DNA biosensors for sequence recognition. *Anal. Lett.*, **2005**, *38*, 2597-2623.

[129] Nirmal, P.; Kavita, A.; Surinder, P. S.; Manoj, K. P.; Harpal, S.; Bansi, D. M. Polypyrrole-polyvinyl sulphonate film based disposable nucleic acid biosensor. *Anal. Chim. Acta*, **2007**, *589*, 6-13.

[130] Nirmal, P.; Sumana, G.; Kavita, A.; Harpal, S.; Malhotra, B.D. Improved electrochemical nucleic acid biosensor based on polyaniline-polyvinyl sulphonate. *Talanta*, **2008**, *74*, 1337-43.

[131] Santandreu, M.; Sole, S.; Fabregas, E.; Alegret, S. Development of electrochemical immunosensing systems with renewable surfaces. *Biosens. Bioelectron*, **1998**, *13*, 7-17.

[132] Kandimalla, V.B.; Neeta, N.S.; Karanth, N.G.; Thakur, M.S.; Roshini, K.R.; Rani, B.E.A.; Pash, A.; Karanth, N.G.K. Regeneration of ethyl parathion antibodies for repeated use in immunosensor: a study on dissociation of antigens from antibodies. *Biosens. Bioelectron*, **2004**, *20*, 903-06.

[133] Sardinha, J.P.M.; Gil, M.H.; Mercader, J.V.; Montoya, A. Enzyme-linked immunofiltration assay used in the screening of solid supports and immunoreagents for the development of an azinphosmethyl flow immunosensor. *J. Immunol. Methods*, **2002**, *260*, 173-182.

[134] Mallat, E.; Barcel´o, D.; Barzen, C.; Gauglitz, G.; Abuknesha, R**.** Immunosensors for pesticide determination in natural waters. *Trends Anal. Chem*., **2001**, *20*, 124-132.

[135] Hleli, S.; Martelet, C.; Abdelghani, A.; Burais, N. Atrazine analysis using an impedimetric immunosensor based on mixed biotinylated self-assembled monolayer. *Sens. Actuators B*, **2006**, *113*, 711-17.

Received: February 01, 2010 Revised: May 23, 2010 Accepted: June 30, 2010