

# Electrochemical Biosensors for Determination of Organophosphorus Compounds: Review

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## ABSTRACT

In last few decades there is exponential increase in use of organophosphorus (OP) compounds as pesticides and insecticides leading to adverse effect on human population and live stock. There is a great need to develop portable analytical tools that are amenable for remediation and bioremediation process monitoring, where rapid analysis of large number of samples is essential. Determination of various organophosphorus compounds has been achieved by integrating bio-components with different transducers. The close integration of the biological events with the generation of a signal offers the potential for fabricating compact and easy-to-use analytical tools of high sensitivity and specificity. With the availability of new materials, associated with new sensing techniques has led to remarkable innovations in the design and construction of organophosphorus biosensors. The present review describes the specifications of most of the electrochemical Organophosphorus biosensors reported till date.

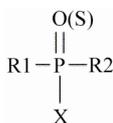
**Keywords:** Organophosphorus Compounds; Acetylcholinesterase; Tyrosinase; Organophosphorus Hydrolase; Electrochemical; Biosensor

## 1. Introduction

Organophosphates (OPs) are usually esters, amides or thiol derivatives of phosphoric, phosphonic, or phosphinic acids, which have general structural formula (**Figure 1**) where R1 and R2 are alkyl-, alkoxy-, alkylthio-, or amido-groups. X is the acyl residue (labile fluorine-, cyano-, substituted or branched aliphatic, aromatic, or heterocyclic groups) [1,2].

Organophosphate (OP) compounds have found wide applications as pesticides and insecticides in agriculture and as chemical warfare agents in military practice. Worldwide, OP compounds account for over 38% of the total pesticides used [3]. Commonly used organophosphates includes parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos and azinphos methyl. Malathion is widely used in agriculture, residential landscaping, public recreation areas and in public health pest control pro-

grams such as mosquito eradication [4]. According to World Health Organization, every year there are three million pesticide poisonings, mostly OP-related, and 200,000 deaths worldwide that are attributed either as self-poisoning or occupational exposure [5]. Besides human exposure, there is also concern that these pesticides could leak into ground and municipal water supplies and pollute surrounding environment. Reports in the literature have expressed concern over exposure to non target organisms such as birds and fish, as well as the potential for human exposure from sources such as fresh fruits and vegetables and processed foods. These neurotoxic compounds, which are structurally similar to the nerve gases Soman and Sarin, irreversibly inhibit the enzyme acetylcholine esterase, essential for the functioning of the central nervous system in humans and insects, resulting in the build up of the neurotransmitter acetylcholine which interferes with muscular responses and in vital organs produce serious symptoms and eventually death [6-8]. Effective methods for degradation/disposal of these toxic compounds are needed to ensure that human and environmental health will not be compromised by the continued use of OP-containing pesticides. Analytical tools to properly monitor the food quality, control any treatment of water may be adopted Laboratory-based methods which are commonly used for de-



**Figure 1. General structure of organophosphorus compounds.**

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tection and measurement of OP pesticide residues include gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis [9,10]. The bioanalytical methods primarily include assays based on enzyme inhibition and immunoassay [11, 12]. Enzyme linked immunosorbant assays (ELISA) are quite sensitive to specific compounds such as ethylparathion or fenitrothion but, like most immunoassays require multiple incubations and generate contaminated plates, tubes, etc. In addition, the characteristics of cholinesterase-based assays and immunoassays for OP pesticides are not well suited to process control monitoring applications as these are typically expensive and time-consuming, further more requires trained man power. Also, laboratory-based methods are not amenable to remediation and bioremediation process monitoring where rapid analysis of large number of samples is essential. Organophosphorus hydrolase (OPH) catalyzes the hydrolysis of a wide range of OP pesticides [13]. The hydrolysis involves a pH change, as well as electroactive species generation. OPH-based assays respond to OP compounds as enzyme substrates rather than inhibitors or antigens this is not the case with acetylcholine esterase. Consequently, these assays can be reversible and require only the analyte of interest. However this method has disadvantages that it employs the free enzymes which can be used once only and the measurement is based on change in pH which limits its sensitivity. Biosensing approach was used to overcome problems of onsite monitoring sensitivity, reliability and ability to screen large number of samples.

## 2. Electrochemical Biosensor

The working of electrochemical biosensors is mainly based on the use of a biological component/bio-receptor element retained in direct contact with an electrochemically active transducer (electrode) to obtain an analytically useful signal by coupling biochemical and electrochemical interactions [14]. The principle of electrochemical sensors is that when an electro-active analyte is subjected to fixed or varying potential of some predefined patterns causes oxidation or reduction of analyte on the working electrode surface, which leads to the generation of an electrochemically measurable signal by the variation on electron fluxes. This signal can be measured by the electrochemical detector.

### 2.1. Electrochemical OP Biosensor Based on Enzyme Inhibition Process

Biosensors based on enzyme inhibition have found wide application for detection of toxic analyte (e.g., OP pesticides) which inhibit the functional activity of the enzyme. By determining the differences in enzyme activity with or without the presence of an inhibitor form the basis of analyte detection, according to the Equation (1):

$$I\% = \left[ \frac{(A_0 - A_i)}{A_0} \right] \times 100 \quad (1)$$

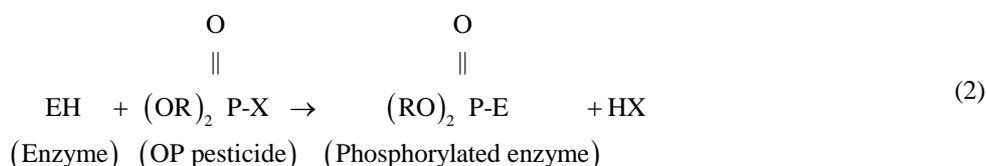
where  $A_0$  is the activity without an inhibitor, and  $A_i$  is with an inhibitor. The linear range is usually comprised between 20% and 80% of inhibition and the detection limit is usually defined as the amount of inhibitor which gives the decrease 20% of inhibition [15].

#### 2.1.1. Use of Acetylcholinesterase (AChE) Enzyme for Preparation of OP Biosensors

The enzyme inhibition-based biosensors for the determination of OP pesticides is described by the following mechanism (2) [16].

Phosphorylated AChE enzymes has lower affinity for the substrate (Acetylcholine) called enzyme inhibition and the degree of inhibition is proportional to the concentration of OP compounds in the sample. Acetylcholinesterase (AChE) inhibition test, using AChE modified amperometric transducers is based on the measure of *para*-Aminophenol produced by hydrolysis of *p*-Aminophenyl acetate, or hydrogen peroxide generated as a result of the oxidation of choline produced from acetylcholine hydrolysis in the presence of choline oxidase. The inhibition of AChE enzyme due to the presence of OP compounds results in reduced reagent consumption and products release is correspondingly detected applying electrochemical techniques and is correlated to the OP pesticides concentration.

AChE enzyme was used in combination with different types of supports for the fabrication of bio-sensing devices [17-34]. **Table 1** summarizes the characteristics of different AChE-based biosensors. Although, sensitive biosensors based on AChE inhibition have few limitations: 1) since ChE is inhibited by neurotoxins which include not only OP pesticides but also carbamate pesticides and many other compounds, these analytical tools, are not selective and cannot be used for quantitation of either an individual or a class of pesticides which may be required to monitor detoxification processes, for example, detoxi-



**Table 1. Characteristics of electrochemical Acetylcholinesterase-based biosensors for OP pesticides detection.**

Sr. No.	Target analyte	Detection technique	Enzyme immobilization technique	Electrode/transducer	Linearity range (M)	Detection limit	Ref.
1.	Paraoxon	Amperometry	Adsorption	AuNPs, graphene oxide nanosheets	ND	$10^{-13}$	[34]
2.	Chlorpyrifos oxon	Amperometry	Entrapment	7,7,8,8-tetracyanoquinodimethane	$6 \times 10^{-9}$ - $2.4 \times 10^{-9}$	$6 \times 10^{-9}$	[39]
3.	Chloropyrifos	Amperometry	Covalence	ZnS NPs Au	$1.5 \times 10^{-9}$ - $4 \times 10^{-8}$	ND	[37]
4.	Paraoxon	Amperometry	Affinity	MWCNT	$3.6 \times 10^{-14}$ - $3.6 \times 10^{-11}$	$5 \times 10^{-15}$	[40]
5.	Chlorpyrifos oxon	CV	Entrapment	PEDOT:PSS	ND	$4 \times 10^{-9}$	[38]
6.	Chloropyrifos	SWV	Cross-linking	SWCNT	$10^{-11}$ - $10^{-6}$	$10^{-12}$	[36]
7.	Chloropyrifos	CV	Covalent binding	Exfoliated graphite nanoplatelets	ND	$1.58 \times 10^{-10}$	[35]
8.	Paraoxon	Amperometry	Entrapment	-	$1.3 \times 10^{-7}$ - $5 \times 10^{-6}$	$3.5 \times 10^{-2}$	[41]
9.	Paraoxon	Amperometry	Cross-linking	CoPc-Prussian Blue	$7.3 \times 10^{-9}$ - $1.8 \times 10^{-8}$	$7.3 \times 10^{-9}$	[42]
10.	Methyl paraoxon	Amperometry	Entrapment	CoPc	$2 \times 10^{-9}$ - $4 \times 10^{-6}$	$2.6 \times 10^{-9}$	[43]
11.	Triazophos	Amperometry	Adsorption	MWCNT	$3 \times 10^{-8}$ - $7.8 \times 10^{-6}$	$10^{-8}$	[44]
12.	Dichlorvos	Amperometry	Adsorption	-	ND	$10^{-10}$	[45]
13.	Dichlorvos	Amperometry	Entrapment	CoPc	ND	$7 \times 10^{-12}$	[46]
14.	Dichlorvos	Amperometry	Adsorption	-	Up to $10^{-16}$	$10^{-17}$	[47]
15.	Dichlorvos	Amperometry	Cross-linking	Prussian blue	$4.52 \times 10^{-11}$ - $4.52 \times 10^{-8}$	$1.13 \times 10^{-11}$	[48]
16.	Trichlorfon	Amperometry	Adsorption	TiO <sub>2</sub> and PbO <sub>2</sub> particles	$10^{-8}$ - $2 \times 10^{-5}$	$10^{-10}$	[49]
17.	Monocrotophos	Amperometry	Adsorption	AuNPs	$4.5 \times 10^{-9}$ - $4.5 \times 10^{-6}$	$2.7 \times 10^{-9}$	[50]
18.	Monocrotophos	Amperometry	Covalent binding	AuNPs-QDs	$4.5 \times 10^{-9}$ - $4.5 \times 10^{-6}$	$1.3 \times 10^{-9}$	[51]
19.	Acephate	FET	Affinity	CNTs	ND	$5.45 \times 10^{-14}$	[52]
20.	Dimethoate	Amperometry	Adsorption	CNTs, zirconia NPs, Au colloid coated Fe <sub>3</sub> O <sub>4</sub> magnetic NPs, Prussian blue	$4.4 \times 10^{-6}$ - $4.4 \times 10^{-2}$	$2.4 \times 10^{-6}$	[29]
21.	Chlorphenvinphos	Amperometry	-	CNTs	$4.90 \times 10^{-7}$ - $7.46 \times 10^{-6}$	$1.15 \times 10^{-7}$	[30]
22.	Malathion	Amperometry	Covalent binding	Fe <sub>3</sub> O <sub>4</sub> NP, c-MWCNT, Au	$10^{-10}$ - $4 \times 10^{-8}$	$10^{-10}$	[32]
23.	Chlorpyrifosoxon	CV and amperometry	Entrapment	PEDOT		$1 \times 10^{-10}$	[33]

fication of OP pesticides. 2) These protocols involve multiple steps requiring measurement of the uninhibited activity of ChE, followed by incubation of the sensor with the analyte sample for 10 - 15 min (and even longer for good sensitivity) and the measurement of the ChE again to determine the degree of inhibition. A final step of re-activation/regeneration, which in many cases is partial and in some cases not possible due to irreversible inhibition, is necessary if the electrode has to be reused.

### 2.1.2. Tyrosine Based OP Biosensor

Tyrosinase through its cresolate activity catalyses the o-hydroxylation of monophenol to o-diphenol, which is further to o-quinone by its catecholase activity. Tyrosi-

nase activity is inhibited by carbamates pesticides and atrazine that lowers the sensitivity of tyrosinase-based biosensors. The Tyrosinase enzyme is inherently unstable and is responsible for reducing the lifetime of the tyrosinase-based biosensors. However, tyrosinase has high optimum temperatures and there is no effect of organic solvents on the activity of enzyme tyrosinase. Numerous electrochemical biosensors based on the inhibition of tyrosinase activity have been reported (Table 2).

### 2.2. OPH Biosensor Based on Direct Catalytic Enzymatic Reaction

In 1970s, *Flavobacterium* sp. ATCC 27551 and *B. di-*

*minuta* were the first OP-degrading bacteria isolated from soil samples [57,58]. Organophosphorus hydrolase (OPH) has broad substrate specificity and is able to hydrolyze a number of OP pesticides such as paraoxon, parathion, coumaphos, diazinon, dursban, methyl parathion [13]. The hydrolysis involves a pH change, as well as electroactive species generation, thus allowing the development of potentiometric and amperometric sensors for OP pesticides quantification [59-65]. The change in pH was measured using a pH electrode and there were drawbacks of sensitivity, calibration. OPH catalyzed hydrolysis of parathion, methyl parathion, paraoxon, fenitrothion, etc. yields 4-nitrophenol. The current of 4-nitrophenol oxidation is proportional to the OP pesticide concentration, is recorded as a biosensor response. OP biosensors have been successfully created using organophosphorous hydrolase as the active component [66]. PTE-immobilized biosensors allow for the direct detection of Ops. However, these biosensors show lower sensitivity values and higher detection limits than cholinesterase-based biosensors. Moreover, they can only detect some Organophosphorus (OP) compounds. The Drawback with such type of sensors is that the potential applied for oxidation of 4-nitrophenol lead to denaturation of the enzyme immobilized on working electrode and thus leads to decrease in activity and reusability. Secondly the potential may oxidize other electro active species that may lead to generation of additional current and false positive results. Characteristics of the other relevant

OPH based electrochemical biosensors based are summarised in **Table 3**.

### 3. Recent Developments in the Fabrication of Electrochemical Biosensors for OP Pesticides Determination

Nanomaterials transducer modification and genetic engineering of the biocomponents are the main strategies to overcome the reported drawbacks of low sensitivity and reusability/regeneration of working electrode. The electro-catalytical properties of the nanostructures includes - their action as electron transfer mediators or electrical wires, large surface to volume ratio, structural robustness, and biocompatibility enhances the use of nano-technological approach in electrochemical biosensors development [74]. Therefore, it gives several advantages like electrode potential lowering, enhancement of the electron transfer rate with no electrode surface fouling, sensitivity increase, stability improvement, and interface functionalization, for developing a bio-sensing system. Various nanomaterials are used for making insoluble support for acetylcholinesterase immobilization in electrochemical biosensors for organophosphorus pesticides determination [75]. By the help of transducer modification with nanomaterials, it gives opportunity to develop biosensors with long storage stability and enables OP pesticides detection in the nanomole-picomole range. The another route leading to increase the biosensors sensitivity, selec-

**Table 2. Characteristics of electrochemical inhibition-based biosensors using tyrosinase for OP pesticides detection.**

Sr. no.	Target analyte	Detection method	Enzyme immobilization technique	Electrode materials	Linearity range (M)	Detection limit (M)	Ref.
1.	Dichlorvos	Amperometry	Cross-linking + entrapment	1,2-naphthoquinone-4-sulfonate (NQS)	Up to $8 \times 10^{-6}$	$6 \times 10^{-8}$	[53]
3.	Methyl parathion	Amperometry	Cross-linking	CoPc	$2.28 \times 10^{-8}$ - $3.8 \times 10^{-7}$	ND	[54]
3.	Diazinon	Amperometry	Cross-linking	CoPc	$6.24 \times 10^{-8}$ - $1.64 \times 10^{-7}$	ND	[54]
4.	Dimethoate	Amperometry	Adsorption	-	$2 \times 10^{-6}$ - $2 \times 10^{-1}$	$10^{-6}$	[55]
5.	Paraoxon	Amperometry	Adsorption	-	$10^{-5}$ - $10^{-2}$	$5 \times 10^{-6}$	[55]
6.	Malathion	Amperometry	Adsorption	-	$10^{-5}$ - $10^{-2}$	$5 \times 10^{-6}$	[55]
7.	Paraoxon	Amperometry	Cross-linking	Prussian blue	$10^{-7}$ - $10^{-6}$	$10^{-7}$	[56]

**Table 3. Characteristics of different OPH-based electrochemical biosensors OP pesticides detection.**

Sr. no.	Target analyte	Detection technique	Immobilization method	Transducer	Linearity range (M)	Detection limit (M)	Ref.
1.	Paraoxon	Amperometry	Covalent binding	SWCNTs	$5 \times 10^{-7}$ - $8.5 \times 10^{-6}$	$10^{-8}$	[67]
2.	Paraoxon	Amperometry	Entrapment	Mesoporous Carbon	$2 \times 10^{-7}$ - $8 \times 10^{-6}$	$1.2 \times 10^{-7}$	[68]
3.	Paraoxon	Amperometry	Entrapment	MWCNTs	Up to $4 \times 10^{-6}$	$15 \times 10^{-8}$	[69]
4.	Paraoxon	Amperometry	Cross-linking	MWCNTs	$5 \times 10^{-7}$ - $2 \times 10^{-6}$	$0.314 \times 10^{-6}$	[70]
5.	Ethyl Parathion	Amperometry	Covalent binding	-	ND	$<3.4 \times 10^{-9}$	[71]
6.	Methyl Parathion	Amperometry	Covalent binding	AuNPs-MWCNTs-QDs	$1.9 \times 10^{-8}$ - $7.6 \times 10^{-7}$	$3.8 \times 10^{-9}$	[72]
7.	Parathion	Amperometry	Cross-linking	CNTs	$2 \times 10^{-9}$ - $4 \times 10^{-8}$	$15 \times 10^{-9}$	[73]

tivity and stability involves the incorporation of tailor designed biorecognition elements in the biosensing platform. Increased bio-recognition element affinity for the target analyte favoring the accessibility of the active site, enhanced electron transfer, and oriented or more stable immobilization can be achieved by appropriate site-directed mutagenesis [76]. Genetically modified enzymes such as AchE, are extensively used in inhibition based biosensors for OP pesticides determination [33], allowing attaining LOD as low as  $10^{-17}$  M [47].

#### 4. Conclusion

Electrochemical biosensors have been found to be suitable for the monitoring of OP compounds. Signal magnification and miniaturization have been achieved by the innovation in fabrication techniques with the use of new materials. With the discovery of new mediators, it is possible to build up an electronic interface between a redox enzyme and transducer for improved signal transmission. From decades variety of prototype have been successfully develop to monitor the conc. of OP compounds. There is a great need for commercial exploitation of the technology for development of portable devices that can be used for field monitoring by untrained manpower.

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