Review Article

Acetylcholinesterase Biosensors for Electrochemical Detection of Organophosphorus Compounds: A Review

Vikas Dhull, 1 Anjum Gahlaut, 2 Neeraj Dilbaghi, 1 and Vikas Hooda 2

1 Department of Bio & Nanotechnology, Guru Jambeshwar University of Science & Technology, Hisar 125001, India
2 Centre for Biotechnology, Maharshi Dayanand University, Rohtak 124001, India

Correspondence should be addressed to Vikas Hooda; vikas.cbtmdu@gmail.com

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The exponentially growing population, with limited resources, has exerted an intense pressure on the agriculture sector. In order to achieve high productivity the use of pesticide has increased up to many folds. These pesticides contain organophosphorus (OP) toxic compounds which interfere with the proper functioning of enzyme acetylcholinesterase (AChE) and finally affect the central nervous system (CNS). So, there is a need for routine, continuous, on spot detection of OP compounds which are the main limitations associated with conventional analytical methods. AChE based enzymatic biosensors have been reported by researchers as the most promising tool for analysis of pesticide level to control toxicity and for environment conservation. The present review summarises AChE based biosensors by discussing their characteristic features in terms of fabrication, detection limit, linearity range, time of incubation, and storage stability. Use of nanoparticles in recently reported fabrication strategies has improved the efficiency of biosensors to a great extent making them more reliable and robust.

1. Introduction

At present pesticides play a major role in agriculture. Pesticides have the insecticidal property due to which they are in great use [1, 2]. But human health and the surroundings are affected by these pesticides as they contain the toxic compounds. These toxic compounds are hazardous as they can accumulate in grains, vegetables, fruits, and so forth, percolate in soil, and finally lead to water contamination [3, 4]. The concentration of these toxic compounds in the environment is increasing day by day with an exponential rate. Organophosphorus (OP) constitutes one of the important classes of toxic compounds which can cause headache, drowsiness, confusion, depression, irritability, disorientation, impaired memory and concentration, speech difficulties, eye pain, abdominal pain, convulsions, respiratory failure, and serious neurological disorders [5–10]. The EPA lists organophosphates as very highly toxic to bees, wildlife, and humans [1]. These OP pesticides inhibit the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) which is involved in the proper functioning of the central nervous system (CNS) of the humans. Due to this inhibition of the enzyme AChE, acetylcholine (ACh) neurotransmitter accumulates in the body which interferes with the muscular responses and finally leads to respiratory problems, myocardial malfunctioning, and even death [11, 12]. The toxicity of different pesticides depends upon the chemical structure of the pesticides [12, 13]. The repeated low level exposure to OP compounds leads to the acute effect on the health of humans. The contamination of soil and food due to these pesticides has caused a serious concern, so it is necessary to monitor their increasing concentration in the food products of daily use. Soil is known to be a natural purifier in which the OP pesticides along with water interact with the soil particles and do not contaminate ground water, but by the time some of the OP pesticides come forward such as organochlorine pesticides which can even percolate even through the soil and contaminate both ground and surface water. Many rules and regulations have been made on the international level to reduce the contamination of ground and surface water. Regulatory limits and the guideline levels are also there for permissible residues in drinking water [14]. It is necessary to develop the methods which are fast, sensitive, and reliable for the detection of OP pesticides
in fruits, vegetables, water, and so forth [15]. Conventional analytical methods to monitor the concentration of these acute toxic compounds include capillary electrophoresis [16], colorimetry [17], gas chromatography (GC) [18], mass spectrometry (MS) [19], thin layer chromatography [20, 21], and high performance liquid chromatography (HPLC) [22]. The above said methods have some limitations, that is, sample preparation which is hectic and time consuming; requiring expensive equipments and trained manpower; less economical; and so forth. To overcome the above problems, development of biosensor is being encouraged. They are simple, sensitive, of low developmental cost, and user friendly; a normal person can handle it easily.

The present review describes and discusses the use of AChE biosensors for detection of OP compounds and measurement of toxicity level in different samples.

2. AChE Based Catalysis

AChE belongs to the family of carboxylesterase (EC number 3.1.1.7.). It is serine protease and stabilises level of acetylcholine (neurotransmitter) by catalysing the conversion of acetylcholine to thiocholine. AChE is concentrated at neuromuscular junctions and cholinergic brain synapses. When the enzyme is present in the active form it terminates synaptic transmission. AChE is highly efficient and catalyses the breakdown of ACh in microseconds keeping the synaptic cleft clear as to avoid the collision of the messages. AChE has two active subsites, anionic and esteratic subsite. Acetylcholine mediates messages between the nerves which is responsible for muscle contraction. When ACh is released from the nerve into the synaptic cleft, it got recognised by ACh receptors present on the postsynaptic membrane which further transmits signal. Along with the ACh receptors AChE is also present on the postsynaptic membrane which helps in the termination of the signal transmission by hydrolysing ACh. On hydrolysis, ACh split into two products one is choline and the other is acetic-acid. Choline and acetic-acid are recycled by the body to again form acetylcholine to maintain the reserves of neurotransmitters so that they can be used by the body again during the time of need. In the presence of inhibitor (OP compound), which forms covalent bond with serine present on the active site of AChE, leads to inactivation of the enzyme [54, 55], and the muscles involved do not relax, leading to paralytic conditions. The intensity of inhibition of AChE is proportional to the concentration of OP compound, that is, inhibitor, and is also exploited as principle of detection method for concentration of OP compounds [55–58].

3. Basic Principle of Biosensors

Biosensor comprises basically of three elements, that is, biological recognition element, transducer, and signal detector as shown in Figure 1. The biological recognition element must be extremely specific to the analyte for the accurate detection of the analyte in different samples. As recognition element and analyte come in close proximity to each other the chemical changes take place in the form of the generation of electroactive species, reduced forms of by-products, consumption of oxygen, and so forth [59]. These changes are detected and displayed on controlling system.

3.1. Principle of OP Biosensor Based on Inhibition Mechanism of AChE

The sensitivity of biosensor relies on the biorecognition layer which catalyses the reaction. The product/by-product further or itself acts as signal which is directly or inversely proportional to the analyte concentration. In the case of AChE inhibition based OP biosensors, the signal generated is inversely proportional to the concentration of OP compound or, in other terms, we can say that increased concentration of OP compound leads to weak signals. The AChE biosensor basically works on the inhibition effect. The biosensor in which the AChE is used as the biorecognition element can detect the toxic organophosphates along with the others such as carbamate pesticides, nerve agents, and several other natural toxins [60, 61]. Some drugs can also be detected with the help of such biosensors [62]. If the inhibitor is not present in the sample then the acetylthiocholine will be converted into the thiocytone and the acetic-acid. as shown in Scheme 1. But if the inhibitor is present in the sample...
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Figure 2: Principle of AChE inhibition-based OP biosensor.

Figure 3: Different supports available for fabrication of working electrode.

then the concentration of thiocholine is decreased or no thiocholine and acetic-acid is produced, in other words it completely inhibits the conversion as shown in Figure 2 [63]. Under the influence of applied voltage thiocholine is oxidised. The anodic oxidation current is inversely proportional to the toxic compound present in the sample and the time of exposure.

In the beginning, AChE biosensors were not considered as reliable tools, but with time the advances in fabrication strategies and methods of enzyme purification and its stabilization have overcome the drawbacks related to accuracy, sensitivity, and reliability [64].

4. Fabrication of AChE Based OP Biosensor

In AChE biosensor the working electrode is prepared by attachment of enzyme on different supports. The supports may be matrices, screen-printed electrodes, semiconductors such as Quantum dots (QD), nanomaterial, and so forth [127], as shown in Figure 3. After immobilization of enzyme onto a particular support, conformational changes take place which finally affect the sensitivity, stability, response time, and reproducibility. A variety of methods are available for immobilization of enzymes including physical adsorption, physical entrapment, covalent coupling, self-assembly monolayer, oriented immobilization, and electropolymerisation. Physical adsorption includes the formation of weak bonds such as the Van der Waals forces, and the electrostatic interactions take place between the enzyme and the support that has an advantage of retaining the activity of immobilized enzyme and method is economical. The drawback associated with this method is the leakage of enzyme [79]. In physical entrapment, AChE enzyme is confined within the gel, the matrices, or in the membranes and used for fabrication of working electrode. This is a one-step procedure which is carried out at low temperature, is simple and cheap, without hampering the activity to enzyme. This method also suffers from leaching of enzyme, nonspecific immobilization, and lower reproducibility. In covalent coupling, stable covalent bond is formed between the support and the enzyme that prevents leaching of enzyme, enzyme is in direct availability for interaction with the analyte that further leads to quick response time. But this method involves a high amount of
enzyme usage, is prone to denaturation, is also expensive, and involves complex procedures [42, 96]. In case of self-assembled monolayer (SAM) the molecules are organised in the form of monolayer. These molecules have the head group and also a tail group having functional groups; head group has affinity towards the substrate. This layer is easy to prepare, molecules are present in the ordered manner, and size is also within the range of nanoscale. Drawbacks of this method are includes difficulties in reproduction and fouling of electrode takes place with time due to the weakening of interaction between the enzyme and the electrode [115, 116]. Oriented immobilization is among one of the new methods which can be used. In this method the particular functional groups of the enzymes are exploited and it is possible to orient the active site of the enzyme towards the analyte. This technique requires less quantity of enzyme with specific control over the orientation [117]. Electropolymerization is also one of the possible methods for the immobilization of the AChE enzyme in which the electric field is used for the polymerization.

4.1. Membranes Used in Fabrication of OP Biosensor. In membrane based AChE biosensors the enzyme is immobilized on the suitable matrices. The membranes which are used as support for immobilization can be natural or artificial. The enzyme is confined to the semipermeable membrane which will allow the passage of the substrate through it. The sensitivity and the selectivity of the membrane based biosensors can be enhanced due to the biocompatibility of the artificial membranes. Different supports have been used for the immobilization of enzyme (Table 1), such as nylon and cellulose nitrate [23], glass/sol-gel/polyvinylidene fluoride [24], hybrid mesoporous silica [25], poly-(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) (PAN) [26, 27], cellophane [28], poly(2-hydroxyethyl methacrylate) membrane [29], polyvinyl alcohol(PVA)/SbQ [30], polyacrylamide [31], bio-immunodyne membrane [118], Si,N,Ti layer [32], pore glass/H+ membrane electrode [33], and hybond N+ membrane [34]. The artificial membranes are selective for the different biomolecules, and as they are highly flexible the response can be enhanced. Membranes are durable and stable on a wide range of pH. The above biosensors suffer from the problem of membrane fouling. The pores of semipermeable membranes are blocked which may lead to hindrance in the passage of solute.

4.2. Polymers Used as Immobilization Support. Polymers can also be used as the support for the enzyme immobilization. The physical and chemical properties of the polymers vary in the wide range which can be exploited for the sensor development [119]. Since polymer supports are flexible, biologically compatible and of low cost, they have advantage over the other supports. They can be used as free standing film for the biosensor fabrication [120].

4.2.2. Conducting Polymer Matrices Used for Enzyme Immobilization. The conducting polymers are the polymers which are synthesized by the chemical and the electrochemical method. The properties of these polymers can easily be adjusted according to the need such as the thickness of film, functionalization, conductivity, and so forth. They can also be used for the enzyme entrapment during electropolymerization and used in the uniform covering of the electrode surface having substrate of any shape and size with the help of the polymer film [122, 123]. Different supports which are used for immobilization of enzymes are (Table 3) poly-(acrylamide)/pH electrode [31], polyethylenimine (PEI)/GCE [41], PEI/SPE [42], mercapto-benzothiazole/polyaniline (PANI)/Au electrode [43], PANI/CNTs coated with single stranded DNA (ssDNA)/Au electrode [44], AuNP-polypyrrole (PPy) nanowire/GCE [45], PPy and PANI copolymer dopped MWNTs/GCE [46], Silk fibroin matrix [47], CS/ALB/GCE [48], PB/GCE [49], GnPs/Chitosan/GCE [50], polymeric enzyme electrode [51], ZrO2/SPE [52], and Gold (Au) nanoparticles/polymethylmethacrylate chloride) (PDDA) protected Prussian blue (PB) matrix [53]. The conducting polymers suffer from demerits of high cost, difficult in processing, lack of mechanical stability after doping, difficult to fabricate, short life span, and so forth.

4.3. Sol-Gel Base AChE Immobilization. Sol-gel is one of the important supports which can be used for the enzyme immobilization. The first and foremost important property of the sol-gel support is that the pore size can be adjusted according to the need. They are also chemically inert, do not show swelling in the aqueous medium, and have photochemical and thermal stability. The antibodies and the enzymes can especially be immobilized and do not allow the leakage of the enzyme in the medium. Some of the accountable demerits include denaturation of biomolecules taking place at high acidic condition and/or high alcohol concentration.
| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|----------------------------|--------------------------|-----------|-----------------------------|--------------------------|--------------------------|-----------|
| Potentiometric    | Nylon and cellulose nitrate/pH electrode | Crosslinking | 0.038 μM 0.077 μM | 50 × 10⁻³–2.5 × 10⁻¹ 50 × 10⁻³–2.5 × 10⁻¹ μM | Trichlorfon, Co-Ral | 15 | 30 15 | [23] |
| Fiber-optic       | Glass/sol-gel/polyvinyl | Crosslinking | 0.53 μM and 0.023 μM | 0.54–3.98 and 0.022–0.13 μM | Carbaryl, dichlorvos | 10 | 21 | [24] |
| Amperometric      | Hybrid mesoporous silica/Pt electrode | Entrapment | 1.2 × 10⁻³ μM 1.0 × 10⁻¹–0.3 μM | DZN-oxon | 15 | 80 | [25] |
| Amperometric      | MWCNTs/PAN/Pt electrode | Affinity bonds using concanavalin A | 5.0 × 10⁻⁹ μM | 3.6 × 10⁻⁷–3.6 × 10⁻⁵ μM | Paraoxon | 20 | 120 | [26] |
| Amperometric      | PAN/AuNPs/Pt electrode | Covalent Bonding | 0.026 × 10⁻⁵ μM | 3.6 × 10⁻⁷–3.6 × 10⁻⁴ μM | Paraoxon | 20 | 30 | [27] |
| Amperometric      | Cellophane/AuE | Crosslinking | 1.45 μM | 1.45–7.26 μM | Paraoxon | 15 | NR | [28] |
| Dissolved Oxygen metric | Poly(2-hydroxyethyl metacrylate)/oxygen electrode | Entrapment | 0.1195 μM 0.05–2.62 μM | Aldicarb | 5 | 2 | [29] |
| Amperometric      | PVA-SbQ/Pt electrode | Entrainement | 7.2 × 10⁻⁵, 0.049 μM | NR | Paraoxon, thifensulfuron methyl | 30 | 30 | [30] |
| Amperometric      | Polyacrylamide/pH electrode | Crosslinking | 3.62 × 10⁻⁵ μM | NR | Dichlorvos | 30 | 50 | [31] |
| Conductometric    | Si₃N₄/Ti layer | Crosslinking | 10 ppb | NR | Zn⁺, Cd⁺ | 30 | 20 | [32] |
| Potentiometric    | Pore glass/H⁺ electrode | Crosslinking | 2 × 10⁻¹⁰ M | 10⁻¹¹–10⁻⁸ M | OP compounds | 30 | | [33] |

**Note:** NR: not reported.

**TABLE 1:** OP biosensors based on membrane immobilized AChE.
Table 2: OP biosensors based on nonconducting polymer immobilized AChE.

| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|-----------------------------|-------------------------|-----------|--------------------------|--------------------------|---------------------------|-----------|
| Amperometric      | MWCNTs/PAN/Pt electrode | Affinity Bonding | $5.0 \times 10^{-5} \mu M$ | $3.6 \times 10^{-3}$–$3.6 \times 10^{-5} \mu M$ | Paraoxon | 20 | 120 | [26] |
| Amperometric      | PAN/AuNPs/Pt electrode | Covalent Bonding | $0.026 \times 10^{-5} \mu M$ | $3.6 \times 10^{-7}$–$3.6 \times 10^{-4} \mu M$ | Paraoxon | 20 | 30 | [27] |
| Amperometric      | MSF/PVA/GCE | Entrapment | $0.2 \times 10^{-3} \mu M$ | $0.2 \times 10^{-3}$–$44.8 \times 10^{-3} \mu M$ | Monocrotophos | 10 | 30 | [35] |
| Amperometric      | PVA/SbQ/SPE | Entrapment | $1.91 \times 10^{-2} \mu M$ | NR | Paraaxon and chlorpyrifos-ethyl oxon | 10 | Nr | [36] |
| Amperometric      | PVA/SbQ/Pt Electrode | Entrapment | $7.2 \times 10^{-3}$, $0.18$, and $0.049 \mu M$ | NR | Paraaxon, maneb, and thifensulfuron methyl | 30 | 30 | [30] |
| Amperometric      | PAMAM-Au/CNTs/GCE | Electrostatic interaction | $4.0 \times 10^{-2} \mu M$ | $4.8 \times 10^{-3}$–$9.0 \times 10^{-2} \mu M$ | Carbofuran | 9 | 21 | [37] |
| Amperometric      | MSF/PVA/GCE | Entrapment | $0.05$ ppb ($0.2$ nM) | $0.05$–$10$ ppb | Monocrotophos | 10 | 30 | [38] |
| Amperometric      | Nylon Net | Covalent bonding | $1.3$–$3.9$ ppb | NR | Paraoxon | 30 | 15–20 | [39] |
| Amperometric      | PBA/SbQ/Pt Electrode | Crosslinking | $25$ ppb–$1.5$ ppm | NR | Chlorpyrifos | 15 | NR | [40] |

Note: NR: not reported.
Table 3: OP biosensors based on conducting polymer immobilized AChE.

| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|------------------------------|-------------------------|-----------|---------------------------|--------------------------|-------------------------|-----------|
| Amperometric      | Polyacrylamide/pH electrode | Crosslinking | $3.62 \times 10^3 \mu M$ | NR        | Dichlorvos                | 30                       | 50                      | [31]      |
| Potentiometric    | PEI/GCE    | Covalent Bonding             | $1.0 \mu M$             | NR        | Dichlorvos                | 10                       | NR                      | [41]      |
| Amperometric      | PEI/SPE    | Non Covalent Bonding          | $1.0 \times 10^{-4} \mu M$ | NR        | Dichlorvos                | 2                        | NR                      | [42]      |
| Amperometric      | Mercaptobenzothiazole/ PANI/Au electrode | Adsorption | $0.48 \times 10^{-3} \mu M$ | NR        | DiazinoFenthion           | 20                       | NR                      | [43]      |
| Electrochemical   | PANI/CNT soDNA/Au electrode | Covalent Bonding | $1.0 \times 10^{-6} \mu M$ | $1.0 \times 10^{-3}$ and $1.0 \mu M$ | Methyl parathion and chlorpyrifos | 15             | 5                       | [44]      |
| Electrochemical   | AuNPs-PPy nanowires GCE | Entrapment | $7.5 \times 10^{-3} \mu M$ | $0.018–0.45$ and $1.89–170 \mu M$ | Methyl parathion | 12                       | 30                      | [45]      |
| Amperometric      | PPY-PANI/MWCNTs/GCE | Adsorption | $3.02 \times 10^{-3} \mu M$ | $0.030–1.51$ and $3.027–75.67 \mu M$ | Malathion | 15                       | 30                      | [46]      |
| Amperometric      | SF/MWNTs/GCE | Adsorption | $5.0 \times 10^{-7} M, 6.0 \times 10^{-8} M$ | $3.5 \times 10^{-6}$ to $2.0 \times 10^{-3} M, 1.0 \times 10^{-7}$ to $3.0 \times 10^{-5} M$ | Methyl parathion, carbaryl | 10             | 4 weeks                 | [47]      |
| Amperometric      | CS/ALB/GCE | Encapsulation | $0.86 \pm 0.098 \mu L$ | $0.25–1.50$ and $1.75–10.00$ | OP pesticides | 10                       | 15                      | [48]      |
| Amperometric      | PB/GCE     | Crosslinking                | $2.5 \text{ng L}^{-1}$ for dichlorvos, $15 \text{ng L}^{-1}$ for omethoate, $5 \text{ng L}^{-1}$ for trichlorfon, and $10 \text{ng L}^{-1}$ for phoxim. | $10 \text{ng L}^{-1}$–1 ng L$^{-1}$ for dichlorvos | Methyl parathion, omethoate, trichlorfon, phoxim | 10             | [49]      |
| Voltammetric      | GnP/Chitosan/GCE | Covalent Bonding | $1.58 \times 10^{-10} \mu M$ | NR        | Chlortpyrifos              | 10                       | 10                      | [50]      |
| Potentiometric    | Polymeric enzyme electrode | Entrapment | $0–10$ ppb | NR        | OP pesticides              | 2                        | NR                      | [51]      |
| Electrochemical   | ZrO$_2$-SPE | Screen printing | $0.02$ nM | $0.05$ nM to $10$ nM | OP compound | 40                       | NR                      | [52]      |
| Amperometric      | Au-PDDA-PB matrix | Covalent Bonding | $0.8$ pg/mL | $1.0$–$1000$ pg/mL and $1.0$–$10$ ng/mL | Monocrotophos | 10                       | 30                      | [53]      |

Note: NR: not reported.
The protocols used for the sol-gel film formation are not amenable for coating the curved surfaces of substrates such as optical fibers; sufficient signals require a high level of biomolecules in sol-gel thin films but it is not possible in the case of proteins that are insoluble or aggregate in the alkoxy silane solution. Sol-gel supports used for immobilization of enzyme (Table 4) are sol-gel/TMOS [65], sol-gel/glass [66], silica sol-gel (SiSG) [67], TMOS/sol-gel [68, 69], chromoionophore/sol-gel [70], Al2O3/sol-gel [71], sol-gel matrix/TCNQ [72], AuNPs-SiSG [73], alumina/sol-gel [74], sol-gel/bromothymol blue [75], Zn(oxide)/sol-gel [76], Si/sol-gel [77], and sol-gel/carbon electrode [78].

4.4. Screen Printing Technique. Screen-printing involves the immobilization of the biological molecules or the biological receptor in their active form. Due to the binding of the molecule in the active form, the analytical changes take place which will affect the sensitivity and the performance of the sensor developed. The necessary action must be taken for the enhancement of the selectivity, sensitivity, exposure time, and so forth. Supports used for immobilization of enzyme (Table 5) are TMOS/sol-gel/SPE [68], Al2O3/sol-gel/SPE [71], sol-gel/TCNQ/modified SPE [72], SPE/TCNQ/Graphite electrode [79], CoPC/SPE [80], phenylenediamine/carbon/CoPC/SPE [81], graphite-epoxy/SPE [82], glutaraldehyde vapour/SPE [83], PVA-SbQ polymer/SPE [36], SWCNT-CoPC/SPE [84], TCNQ modified graphite [85], Au electrode [86], screen printed carbon electrode [87], and PET chip SPE [88]. Screen-printing is unstable, has high cross-sensitivity towards anion, and limited life span.

4.5. Quantum Dot as Immobilization Support for AChE. Quantum dots are highly luminescent photostable fluorophore. QDs are the semiconductor particles that have all the dimensions confined to the nanometre scale [124]. They have been used in biosensors as they have their great size dependent properties and are dimensionally similar with the biological molecules which are used for immobilization [125, 126]. QDs can even be coupled with the variety of biological molecules due to which they are important in the sensing and development of the sensitive sensors. They suffer from demerits such as large size (10 to 30 nm) and blinking behaviour if no emission interrupts longer periods of fluorescence. The supports which are used for the immobilization of the enzymes are (Table 6) supports used for immobilization of enzyme: CdTe QDs/AuNPs/CHIT/GCE [73], CdTe QDs/Au electrode [89], poly(allylamine hydrochloride)/CdTe QDs/glass electrode [90], Mn/ZnSe d dots [91], and CdTe QDs/Au electrode [92].

4.6. Nanomaterial Based AChE Immobilization. To improve the reliability of electrochemical based technique, researchers have been exploring the possibilities of new materials for improving the properties of transducers. Nanoparticles are proving to be a boom in the field of biosensing due to their invaluable properties such as large surface area, high conductivity, good catalytic property, and so forth. The rate of electron transfer is enhanced to a great extent. They can be synthesized in the laboratory and even their particle size can be adjusted according to the need. The carbon nanotubes are in regular use nowadays such as Single Walled Carbon Nanotubes (SWCNTs) and Multiwalled Carbon Nanotubes (MWCNTs). These carbon nanotubes are highly conductive and have large surface area. Different supports used for immobilization of enzyme (Table 7) are AuNPs-CaCO3/Au electrode, Iron(Fe) NP/MWCNTs/Au electrode, FeNP/MWCNTs/indium tin oxide (ITO) electrode, AuNPs/PB/GCE [93], MWCNTs-Au nanocomposites/GCE [94], ZrO2/CHIT/GCE [95], Au-Pt bimetallic NPs/GCE [96], AuNPs/GCE [97], AuNPs-MWCNTs/GCE [98], PB/CHIT/GCE [99], TiO2 graphene/GCE [100], graphite-nanoplatelet CHIT composite/GCE [101], calcium carbonate-CHIT composite/GCE [102], Cds-decorated graphene nanocomposite [103], CHIT-GNPs/Au electrode [92], MWCNTs-CHIT/GCE [104], AuNPs/Au electrode [105], PbO2/TiO2/Ti [106], PB-CHIT/GCE [107], Er-GRO/Nafion [108], SWCNT modified FGE [109], Au-PtNPs/3-aminopropyltriethoxysilanes (APTEs)/GCE [110], CNT web modified GCE [111], PAN-AuNPs [112], CdTe AuNPs Film [113], and SiSG-AuNPs [114].

5. Conclusion and Future Prospects

It is clear from the comprehensive review presented above that the AChE based OP biosensor is an important research field, with lots of applications in environmental monitoring, human health concern, and food industries. With the development of the selective biorecognition elements the high throughput screening of analyte is now possible in a reliable manner in a fraction of seconds. The large number of samples can be screened with ease and accuracy. The oxidising and reducing ability of the biorecognition element has made electrochemical biosensor the most appropriate tool for the detection purpose over the other available methods [127]. The electrochemical biosensors have the unique ability to convert the catalytic signal into the quantifiable digital signal using microfabrication electronics. Nanoparticles are proving to be most eligible in fabrication of different working electrodes. The nanomaterials can be easily synthesized in the laboratory according to the need in respect to their size and dimensions. The conductivity of nanomaterials is high due to efficient electron transfer channels developed with respect to the other supports used. The self-life of the AChE biosensor can also be increased by using nanoparticles based electrodes. A vast variety of working electrodes for the sensor development can be fabricated for the improved detection of OP compounds in different samples. The on spot detection is also an important parameter for the biosensors which is possible due to the screen-printing technology. Screen-printed biosensors can be fabricated in miniaturization form for on-site rapid monitoring of the analyte. But till now the commercialization of the biosensors has not been possible due to the high cost of the enzyme in the market. Less work has been done on the validation of the enzymatic biosensors with respect to the real samples. Many interfering compounds are present in
| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|-----------------------------|-------------------------|-----------|--------------------------|-------------------------|------------------------|-----------|
| Optical           | Sol-gel/TMOS | Encapsulation              | 0.94 μM 42.19 μM        | 3.17–31.48 14.89–998.40 μM | Naled, Mecarbam     | 5                       | 30                     | [65]      |
| Optical           | Sol-gel/Glass | Encapsulation              | 0.098 μM 0.098–0.55 μM   |           | Paraoxon                | 30                      | NR                     | [66]      |
| Amperometric      | Silica sol-gel/SPE | Encapsulation              | 0.024, 0.015, and 0.012 μM | 0.01–0.001 μM | Paraoxon, dichlorvos, and chlorpyrifos-ethyl oxon | 20                      | 6                      | [67]      |
| Amperometric      | TMOS sol-gel/SPE | Encapsulation              | 1.0 × 10⁻³ μM           | 1.0 and 3.0 × 10⁻³ μM | Dichlorvos            | 15                      | NR                     | [68]      |
| Amperometric      | TEOS sol-gel/GCE | Encapsulation              | 0.008 μM 0.008–0.81 μM   |           | Oxydemeton methyl       | 20                      | 21                     | [69]      |
| Optical           | Chromo-ionophore/Sol-gel | Encapsulation              | 2.26 μM 2.26–31.67 μM   |           | Dichlorvos             | 15                      | NR                     | [70]      |
| Amperometric      | Al₂O₃ sol-gel matrix SPE | Adsorption                | 0.01 μM 0.1–80 μM       |           | Dichlorvos             | 15                      | 5                      | [71]      |
| Amperometric      | Sol-gel matrix on TCNQ modified SPE | Entrapment                | 1 × 10⁻³, 8 × 10⁻³, and 2 × 10⁻² μM | NR | Carbaryl, carbofuran, and pirimicard | 20                      | 45                     | [72]      |
| Electrochemical   | AuNPs-SiSG/GCE | Hydrogen bonds             | 0.44 μM NR               |           | Monocrotophos          | 10                      | 30                     | [73]      |
| Amperometric      | Alumina/sol-gel/sonogel composite/Carbon electrode | Encapsulation             | 2.5 × 10⁻⁴ μM           | 0.5 μM   | Chlorpyrifos-ethyl oxon | 10                      | 50                     | [74]      |
| Optical           | Bromothymol blue/sol-gel | Encapsulation             | 0.11 μM 0.14–5.70 μM    |           | Chlorpyrifos          | 8                       | 10                     | [75]      |
| Amperometric      | Zinc oxide/sol-gel/SPE | Encapsulation             | 0.127 μM 0.127–5.00 μM |           | Paraoxon              | 10                      | 90                     | [76]      |
| Amperometric      | Silica/sol-gel/Carbon electrode | Encapsulation           | 3.0 × 10⁻⁴ and 0.47 μM | 3.7 × 10⁻¹–1.8 × 10⁻³ and 0.27–4.09 μM | Methyl parathion and acephate | 20 and 4 | 30                     | [77]      |
| Cyclic Voltametry | Sol-gel/carbon electrode | Entrapment               | 0.04 ppb for parathion, 47 ppb for monocrotophos | 0.1–1.0 ppb | Parathion and monocrotophos | 10                      | NR                     | [78]      |

Note: NR: not reported.
Table 5: OP biosensors based on screen-printed electrodes.

| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|------------------------------|--------------------------|-----------|---------------------------|--------------------------|--------------------------|-----------|
| Amperometric      | TMOS sol-gel/SPE | Encapsulation | $1.0 \times 10^{-2} \, \mu M$ | 1.0 and $3.0 \times 10^{-3} \, \mu M$ | Dichlorvos | 15 | NR | [68] |
| Amperometric      | Al₂O₃ sol-gel/SPE | Adsorption | $0.01 \, \mu M$ | 0.1-80 \, \mu M | Dichlorvos | 15 | 5 | [71] |
| Amperometric      | Sol-gel/TCNQ/SPE | Entrapment | $1 \times 10^{-2}, \, 8 \times 10^{-3}, \text{and} \, 2 \times 10^{-4} \, \mu M$ | NR | Carbaryl, carbofuran, and pirimicard | 20 | 45 | [72] |
| Amperometric      | SPE/TCNQ/Graphite Electrode | Adsorption | $3.0 \times 10^{-6} \, \mu M$ | $5 \times 10^{-2}$-0.2 \, \mu M | Chlorpyrifos-ethyl oxon | 10 | 50 | [79] |
| Amperometric      | CoPC/SPE | Crosslinking | $4.9 \times 10^{-5} \, \mu M$ | 10-5-1.0 \, \mu M | Carbofuran | 15 | NR | [80] |
| Amperometric      | Phenylendiamine/ CoPC SPE | Entrapment | $1 \times 10^{-5}, \, 1 \times 10^{-10}, \text{and} \, 1 \times 10^{-15} \, \mu M$ | $1.0 \times 10^{-2}$ | Dichlorvos, parathion, and azinphos | 10 | 92 | [81] |
| Amperometric      | Graphite-epoxy/SPE | Crosslinking | $1.0 \times 10^{-7}$ and $1.0 \times 10^{-7} \, \mu M$ | NR | Paraoxon and carbofuran | 15 | 5 | [82] |
| Amperometric      | Glutaraldehyde vapour/SPE | Crosslinking | 0.18 \, \mu M | 0.18–54.00 \, \mu M | Paraoxon | 10 | NR | [83] |
| Amperometric      | PVA-SbQ/SPE | Entrapment | $1.91 \times 10^{-7} \, \mu M$ and $1.24 \times 10^{-3} \, \mu M$ | NR | Paraoxon and chlorpyrifos-ethyl oxon | 10 | NR | [36] |
| Amperometric      | SWCNTs-CoPC/SPE | Covalent Bonding | 0.01 and $6.3 \times 10^{-3} \, \mu M$ | $0.018-0.181$ and $6.36 \times 10^{-3}$-0.159 \, \mu M | Paraoxon and malaoxon | 15 | 3 | [84] |
| Amperometric      | TCNQ modified-graphite | Screen printing | 1 ppb | 0–5 \, \times \, \mu M | Methamidophos | 10 | NR | [85] |
| Amperometric      | Gold electrode | Crosslinking | 0.1 mM | 1–10 mM | Paraoxon | 28 | | [86] |
| Amperometric      | Carbon electrode | Covalent Bonding | $10^{-10} \, \mu M$ | NR | Dichlorvos | 60 | NR | [87] |
| Amperometric      | SPE | Copolymerisation | 4 to 7 \, \mu g/L | NR | Dichlorvos, methyl-parathion | 4 | NR | [88] |

Note: NR: not reported.
| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|------------------------------|--------------------------|-----------|---------------------------|--------------------------|--------------------------|-----------|
| Electrochemical   | AuNPs-SiSG/GCE | Hydrogen Bonding | 0.44 μM | NR | Monocrotophos | 10 | 30 | [73] |
| Amperometric      | CdTe QDs/AuNPs/CHIT/GCE | Covalent Bonding | 1.34 μM | 4.4 × 10^{-3}–4.48 and 8.96–67.20 μM | Monocrotophos | 8 | 30 | [89] |
| Optical           | CdTe-QDs/Glass | Electrostatic interaction | 1.05 × 10^{-5} and 4.47 × 10^{-6} μM | 1.0 × 10^{-6}–1.0 and 1.0–0.1 μM | Paraoxon | 15 | 35 | [90] |
| Fluorescence quenching | Mn:ZnSe d-dots | NR | 1.31 × 10^{-11} mol | 4.84 × 10^{-11} to 4.84 × 10^{-6} mol/L | Paraoxon | 10 | NR | [91] |
| Amperometric      | CdTe QDs/Au electrode | Covalent Bonding | 2.98 × 10^{-3} μM | 4.96 × 10^{-7}–2.48 μM | Carbyl | 10 | 30 | [92] |

Note: NR: not reported.
| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|-----------------------------|--------------------------|-----------|---------------------------|--------------------------|---------------------------|-----------|
| Amperometric      | AuNPs/PB/GCE | Surface Adsorption           | $3.5 \times 10^{-9}$ µM  | $4.48 \times 10^{-7}$–$4.48 \times 10^{-2}$ µM | Monocrotophos            | 10                       | 30                       | [93]      |
| Amperometric      | MWCNTs-AuNC/GCE | Hydrophilic adhesion        | $1.81 \times 10^{-7}$ µM | $3.0 \times 10^{-7}$–$3.027$ µM | Malathion                | 8                        | 30                       | [94]      |
| Amperometric      | ZrO$_2$/CHIT/GCE | Surface Adsorption           | $1.3, 5.0 \times 10^{-9}$, and $1.7$ µM | $6.6$–$440, 0.01$–$0.59$, and $8.6$–$520$ µM | Phoxin, malathion, and imethoate | 15                       | 30                       | [95]      |
| Amperometric      | Au-PtNPs/GCE | Crosslinking                 | $50 \times 10^{-4}$, $40 \times 10^{-3}$, and $40$ µM | $50$–$200 \times 10^{-3}$, $1.40$–$50 \times 10^{-3}$, and $40$–$60$ µM | Paraoxon ethyl, sarin, and aldicarb | 25                       | NR                       | [96]      |
| Amperometric      | AuNPs/GCE | Surface Adsorption           | $7.0 \times 10^{-3}$ µM  | $28 \times 10^{-3}$–$170 \times 10^{-3}$ µM | Methamidophos            | 10                       | 7                        | [97]      |
| Amperometric      | AuNPs-MWCNTs/GCE | Surface Adsorption         | $1.0 \times 10^{-3}$ µM  | $0.1 \times 10^{-3}$–$7.0 \times 10^{-3}$ µM | NR                       | 30                       | NR                       | [98]      |
| Amperometric      | PB/CHIT/GCE | Crosslinking                 | $0.113 \times 10^{-4}$, $0.703 \times 10^{-5}$, and $0.33 \times 10^{-3}$ µM | $0.45 \times 10^{-1}$–$0.045$, $0.234 \times 10^{-3}$–$0.046$, and $0.116 \times 10^{-3}$–$0.0394$, and $0.167 \times 10^{-3}$–$0.0355$ µM | Paraoxon and chlorpyrifos-ethyl oxon | 10                       | NR                       | [99]      |
| Amperometric      | TiO$_2$-decorated graphene/GCE | Surface Adsorption         | $1.4 \times 10^{-3}$ µM  | $4.9$–$74.5$ and $74.5$–$9 \times 10^{3}$ µM | Carbyl                    | 3                        | 20                       | [100]     |
| Voltammetric      | Graphite/CHIT/GCE | Covalent Bonding          | $1.58 \times 10^{-4}$ µM  | $1 \times 10^{-3}$–$1$ µM | Chloropyrifos            | 10                       | 10                       | [101]     |
| Voltammetric      | MWCNTs/AuNPs-CHIT/GCE | Surface Adsorption         | $0.01$µM                  | $0.1$–$10$ µM | Monocrotophos            | NR                       | 50                       | [102]     |
| Amperometric      | Cds-decorated graphene nanocomposite | Surface Adsorption         | $3.4 \times 10^{-3}$ µM  | $9.9 \times 10^{-3}$–$9.93$ µM | Carbaryl                | 2                        | 20                       | [103]     |
| Mode of detection | Transducer | Enzyme immobilization method | Minimum detection limit | Linearity | Substrate/enzyme inhibitor | Time of incubation (min) | Storage stability (days) | Reference |
|-------------------|------------|-----------------------------|-------------------------|-----------|----------------------------|-------------------------|--------------------------|-----------|
| Amperometric      | CHIT-GNP/Au electrode | Chemical Adsorption | $0.1 \times 10^{-3}$ $\mu$M | $0.3 \times 10^{-3}$–$60.5 \times 10^{-3}$ $\mu$M | Malathion | 15 | NR | [92] |
| Amperometric      | MWCNTs-CHIT/GCE | Covalent Bonding | NR | NR | Carbaryl, malathion, dimethoate, and monocrotophos | 8 | 30 | [104] |
| Amperometric      | AuNP/Au electrode | Surface Adsorption | $33 \times 10^{-3}$ $\mu$M | $10 \times 10^{-7}$–$135 \times 10^{-3}$ $\mu$M | Carbofuran | 20 | 7 | [105] |
| Amperometric      | PbO$_2$/TiO$_2$/Ti | Surface Adsorption | $0.1 \times 10^{-3}$ $\mu$M | $0.01$–$20$ $\mu$M | Trichlorfon | 10 | 5 | [106] |
| Amperometric      | PB-CHIT/GCE | Covalent Bonding | $3.0 \times 10^{-3}$ $\mu$M | $0.01$–$0.4$ and $1.0$–$5.0$ $\mu$M | Carbaryl | 10 | 30 | [107] |
| Amperometric      | Er-GRO/Nafion | Surface Adsorption | $2.0$ ng mL$^{-1}$ | $5.0$–$100$ ng mL$^{-1}$ and $1.0$–$20$ ng mL$^{-1}$ | Dichlorvos | 10 | 28 | [108] |
| Potentiometric    | SWCNT modified FGE | Crosslinking | $25$–$35$ nM and $15$–$20$ nM for sarin and DFP, respectively | $20$–$60$ nM and $20$–$80$ nM for sarin and DFP, respectively | Sarin and DFP | 5 | 30 | [109] |
| Amperometric      | Au-PtNP/3-APTES/GC electrode | Crosslinking | $150$–$200$ nM, $40$–$50$ nM, and $40$–$60$ $\mu$M for paraoxon ethyl, sarin, and aldicarb | NR | Paraoxon ethyl, sarin, and aldicarb | 10 | NR | [110] |
| Amperometric      | CNT-web modified glassy carbon electrode | Surface Adsorption | 1 nM | $20$–$1000$ nM | Methyl parathion | 20 | NR | [111] |
| Amperometric      | PAN-AuNPs | Covalent Bonding | $7.39 \times 10^{-8}$ g L$^{-1}$ | $10^{-9}$–$10^{-7}$ g L$^{-1}$ | Paraoxon | NR | 20 | [112] |
| Voltammetric      | CdTe-GNP film | Covalent Bonding | $0.3$ ng mL$^{-1}$ | $1$–$1000$ ng mL$^{-1}$ and $2$–$15$ ng mL$^{-1}$ | Monocrotophos | 8 | 30 | [113] |
| Amperometric      | SiSG-AuNPs | Surface adsorption | $0.6$ ng/mL | $0.001$–$1$ $\mu$g/mL and $2$–$15$ $\mu$g/mL | Monocrotophos | 10 | 30 | [114] |

Note: NR: not reported.
the sample and can hamper the sensitivity of the biosensor. The biosensors must be validated to explore the effect of interfering compounds on the pesticide detection.

Conflict of Interests
The author(s) declare(s) that there is no conflict of interests regarding the publication of this paper.

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