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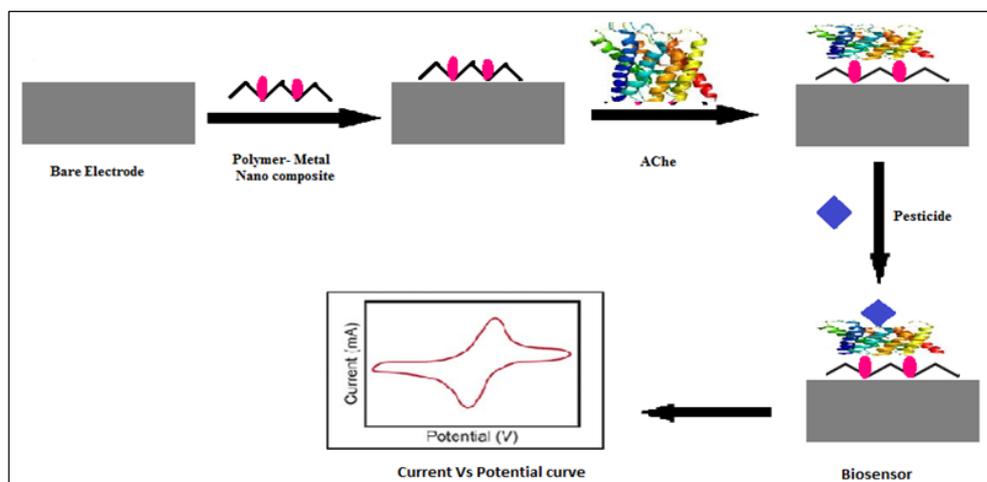
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Electrochemical sensor for the detection of pesticides in environmental sample: A review

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Abstract

Many of the pesticides are acutely toxic and have lasting effect on human health. The primary reason for organophosphate (OP) toxicity is its inhibition of the acetyl cholinesterase (AChE) enzyme. Significant work is reported on electrochemical sensors for OP detection and they have been proven as an inexpensive, simple and versatile analytical tool. They have found a vast range of important applications in the fields of clinical, industrial, environmental and agricultural analyses. Their sensitivity, reproducibility and specific selectivity towards target OP molecules can be improved by applying different coating materials at electrode surface. In this review focus is on the coating materials for the modification of working electrodes surface to use it widely as sensors for electroanalytical strategy for many sensing applications requiring highly compatible and properly functionalized surfaces to anchor the recognition material.



Graphical Abstract: Schematic illustration of the stepwise AChE biosensor fabrication process and immobilized AChE inhibition in pesticide solution

Keywords: organophosphate, acetylcholinesterase, electrochemical sensor

Introduction

Pesticide is an organic toxic substance or mixture of substances used to prevent, destroy, repel, or mitigate insects, bacteria, weeds, nematodes, rodents that causes harm ^[1]. There are six major classes of pesticides including organophosphates (OPs), carbamates, organochlorines, pyrethroid, metal and organometal pesticides ^[2]. They are widely used in agriculture to improve yield and to protect seeds and crops before and after harvesting, leading to an increase in its application over the years but the pesticide residues are contaminating our environment and may enter into the food chain through water, air and soil and cause serious problems to environment and human health ^[1].

Organophosphates pesticides (OPs) are usually esters, amides or thiol derivatives of phosphoric, phosphonic or phosphinic acids. They came into existence in 1932 and some organophosphates due to their acute toxicity were used in 2nd world war as nerve agents ^[3]. OPs are widely used in agriculture for crop protection and pest control due to their high

insecticidal activity, good efficiency and comparatively low persistence in the environment. They are also been used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants and gasoline additives. Some of the organophosphate works as potential insecticides, pesticides which includes parathion, trimesters of phosphates, malathion, chlorpyrifos, diazinon, chlorfenvinphos, dicrotophos, disulfoton, azinphos-methyl, coumaphos, ethion. Methyl parathion (o, o-dimethyl-o-(4-nitrophenyl) phosphorothioate, MP) is an organophosphorous compound widely used for pest control in a large variety of crops including cereals, fruits, coffee, potato and sugarcane [4]. It is used extensively in reducing agricultural losses caused by fruit flies, bugs and other insects. Lethal dosage of MP as declared by WHO is 3 mgkg⁻¹. Monocrotophos (C₇H₁₄NO₅P) an organophosphorus pesticide is chronically toxic to humans through inhalation, oral administration and skin. It is used as chemical warfare, mass genocide, suicidal attempt and accidental exposure [5]. Chlorpyrifos [o,o-diethyl-o-(3,5,6-trichloro-2-pyridinyl) phosphor othioate] (CHL) is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide and nematicide. It possesses low water solubility (1.39 mgL⁻¹) and high soil sorption, 360 to 31,000 depending on soil type and environmental conditions. Chlorpyrifos persists in soil for 60-120 days and produces toxic effects to human beings and animals. CHL get absorbed through skin, gut and pulmonary membranes by ingestion and contact. It is produced by the reaction of 3, 5, 6-trichloropyridin-2-ol (TCP) with O, O-diethyl phosphor rochlorodithioate [6]. CHL is a colorless to white crystalline solid. Chlorpyrifos has a mild mercaptan (thiol) odour, similar to the smell of sulfur compounds found in rotten eggs, onions, garlic and skunks. It is used at the rate of over 50,000 kg per year in Europe [7] and in amounts exceeding 5 million kg per year in US agriculture [8]. CHL effects immune system by disturbing AChE activity in developing foetus, mammalian cell cultures and lead to several neuro developmental disorders in humans and animals [9]. Aquatic species are badly affected by these chemicals, by direct exposure of contaminated preys fishes bio-accumulate these chemicals through food chain [10]. OPs due to their high solubility in water when sprayed get mixed into the soil and then into the underground water which causes acute toxicity in lakes and rivers and they get contaminated through rains and wind, many other organisms are affected due to this. Only 0.1% reaches the specific target which is a matter of concern [11]. To protect human health from this hazard there is a great need to develop selective, sensitive, fast, reliable methods for the detection of OPs. OPs are highly toxic as they efficiently absorbed by inhalation, ingestion and dermal penetration. OPs causes toxicity in Central Nervous System (CNS), they have structural resemblance to nerve gases soman, sarin, tabun and causes irreversible inhibition of acetylcholine sterase (AChE) (catalyse hydrolysis of neurotransmitter acetylcholine). AChE is very important for CNS functioning, muscular responses and for other organs AChE hydrolyses acetylthio choline in nerve junctions to choline and acetic Acid [12]. Inhibition of AChE leads to disruption of nerve impulses transmission in vertebrates, birds, fishes and insects OPs are very harmful for humans, symptoms for OP poisoning are headache, hypertension, hypotension, muscle twitching, nausea, diarrhoea, vomiting, respiratory disorders, myocardial malfunctions and even may lead to death [13]. Many efforts have been made to develop simple, sensitive, convenient, reliable, and effective methods for OPs analysis in environmental samples. Traditional complicated analytical

methods for detection of OP compounds include gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis, mass spectroscopy and thin layer chromatography [14-17]. But these procedures are quite sophisticated and involve expensive instrumentation, have low sensitivity with the large consumption of chemicals, time consuming and require more trained man power. Electrochemical (EC) methods overcome the problems associated with traditional analytical methods as these are cost effective, portable (in situ monitoring), highly sensitive, selective and have simplicity in operation, reusable, require less organic solvents, low sample volume and short analysis time. By EC method miniaturization is possible, which gives a possibility of online monitoring for detection and can be directly operated in environmental samples [18,19]. In the present study Cyclic Voltammetry (CV) and Square wave voltammetry (SWV) will be implemented, these methods involve three electrodes system working, reference and auxillary electrode. CV is an electro analytical technique, widely used to study reversible, irreversible and quasi-reversible redox reaction. In performing an electro analytical study, CV is considered to be the first experimental approach, as it offers rapid location of redox potentials of the electro active species and a convenient evaluation of the effect of media on the redox process involved. A cyclic voltammo gram is obtained by applying a linear sweep potential to the working electrode, due to this a current flows through the analyte that is responsible for either oxidation or reduction of target analyte [20].

The most effective and improved form of pulse voltammetry is SWV. In SWV potential is applied in the form of a pulse superimposed as a staircase wave. The sampling of current occurs both at the end of half oxidation and half reduction potential. The differential current is recorded against the potential which results in a peak shaped voltammogram. SWV is more convenient in comparison to other techniques due to its speed. Extremely fast scan rates has resulted in drastic reduction in time of analysis. It takes few seconds to record the entire voltammogram It is used in the pesticide analysis is a highly sensitive electro analytical technique, ito lower the limit of detection value of the sensor. The major advantages of this technique include fast, selective, sensitivity (10⁻⁹ molL⁻¹) and accuracy [21].

Electrode coating materials for working electrode surface

For the preparation of an enzyme based sensor, attachment of the enzyme on the surface of the WE is an essential step. The WE is developed by allocation of composite on different supports like screen printed electrodes (SPE), matrices, quantum dots (QD) and nano materials. After the interaction between enzyme and electrode surface the reproducibility, response time, sensitivity and stability of the WE gets highly improved. Immobilization of an enzyme on the surface of electrode takes place by different techniques which includes electropolymerisation, physical entrapment, physical adsorption, self-assembly monolayer, covalent coupling and oriented immobilization.

In the process of physical adsorption, the AChE gets simply deposited onto the WE, through weak Vander waal forces. This method causes no damage to enzyme, very simple, economical and not disturb the chemical behaviour of enzyme. Disadvantages are short response time, storage stability as it is sensitive to changes in temperature, pH, ionic strength and enzyme leakage [22].

Physical entrapment is a one step process, which is carried out

at low temperature AChE enzyme is confined within the gel, the matrices, or in the membranes and used for fabrication of We. It is highly economical but it suffers from enzyme leaching, lower reproducibility and non-specific immobilization [23-25]. Covalent coupling of an enzyme is the most widely used procedure. In this enzyme is covalently linked to the electrode surface by covalent bonds which prevents enzyme leakage and it has a short response time but this technique is highly expensive, involves complicated procedures and denaturation problems [26-29]. In self-assembled monolayer (SAM), head and tail groups are present, the former has affinity for substrate and the later have functional groups attached to them. SAM have various advantages like it is highly ordered, can be easily prepared, size is in nano range and a lot of functional groups are attached to its terminal end but in this electrode fouling takes place [30, 31]. Electro polymerization is another method for enzyme immobilization, in this technique polymerisation takes place under the influence of electrical field. It prevents electrode fouling but the demerit associated with this method is its occurrence only on conducting materials.

Metal nanocomposite based electrodes

Metal oxides (Al_2O_3 , SnO_2 , TiO_2 , CuO , RuO_2 , MnO_2 , V_2O_5 and MgO) have been widely used as important electrode materials for the various electrochemical applications including sensors, electronic devices and catalytic applications [32]. As compared to other electrode materials, metal oxides possess uniform size, identical shape and well defined catalytic applications.

For determination of parathion, a nano thin film was synthesized by molecularly imprinted nano- TiO_2 self-assembled electrode method. The linear response of parathion was found in the concentration range 5.0×10^{-8} and 1.0×10^{-5} molL^{-1} , and the L.O.D. was 1.0×10^{-8} molL^{-1} [33]. For parathion detection an EC sensor was also developed by modified GCE with AuNPs and MWCNTs composite with

CV electro deposition method. For the characterisation X-ray diffraction and FES electron microscope methods were employed. It was observed that concentration range for parathion was 6.0×10^{-5} to 5.0×10^{-7} molL^{-1} with L.O.D. of 1.0×10^{-7} molL^{-1} [34].

Cobalt (II) oxide-decorated reduced graphene oxide electrode was reported for monitoring of carbaryl and carbofuran in food samples and L.O.D. value for carbaryl and carbofuran was $7.5 \mu\text{gL}^{-1}$ and $4.2 \mu\text{gL}^{-1}$ respectively [35].

Based on platinum carbon aerogels (PtCAs) composite AChE biosensor with high sensitivity and stability have been developed for detecting OPs. Method employed in the preparation of PtCAs composite involves liquid phase reduction of ethylene glycol and solgel polymerisation methods. Boron Doped Diamond (BDD) electrode was modified for AChE immobilization. Inhibition rate of monocrotophos and methamidophos were linearly proportional to their concentrations in the range of 10^{-11} - 10^{-6} molL^{-1} and L.O.D were found to be 2.7×10^{-12} molL^{-1} (0.6 ppt) for Monocrotophos and 3.1×10^{-13} molL^{-1} (0.05 ppt) for methamidophos [36].

A highly selective and sensitive AChE biosensor has been developed by the modification of GCE with PtNPs, Carboxylic graphene (CGR) and nafion (NF). For adhesion of AChE a hydrophilic surface was offered by the PtNPs-CGR-NF nanocomposites with excellent conductivity, catalysis and biocompatibility. To immobilize AChE Chitosan (CS) was used as a crosslinker and NF membrane imparts protection of AChE biosensor. The AChE showed resemblance to acetylthiocholinechloride (ATCI) and could catalyze the hydrolysis of ATCI. Methyl parathion was detected from 1.0×10^{-13} to 1.0×10^{-8} molL^{-1} with L.O.D of 5×10^{-14} molL^{-1} and detected carbofuran in the linear range from 1.0×10^{-12} to 1.0×10^{-8} molL^{-1} with a detection limit of 5.0×10^{-13} molL^{-1} (Fig. 1). The biosensor exhibited good sensitivity, acceptable stability and reproducibility, thus providing a promising tool for analysis of enzyme inhibitors [37].

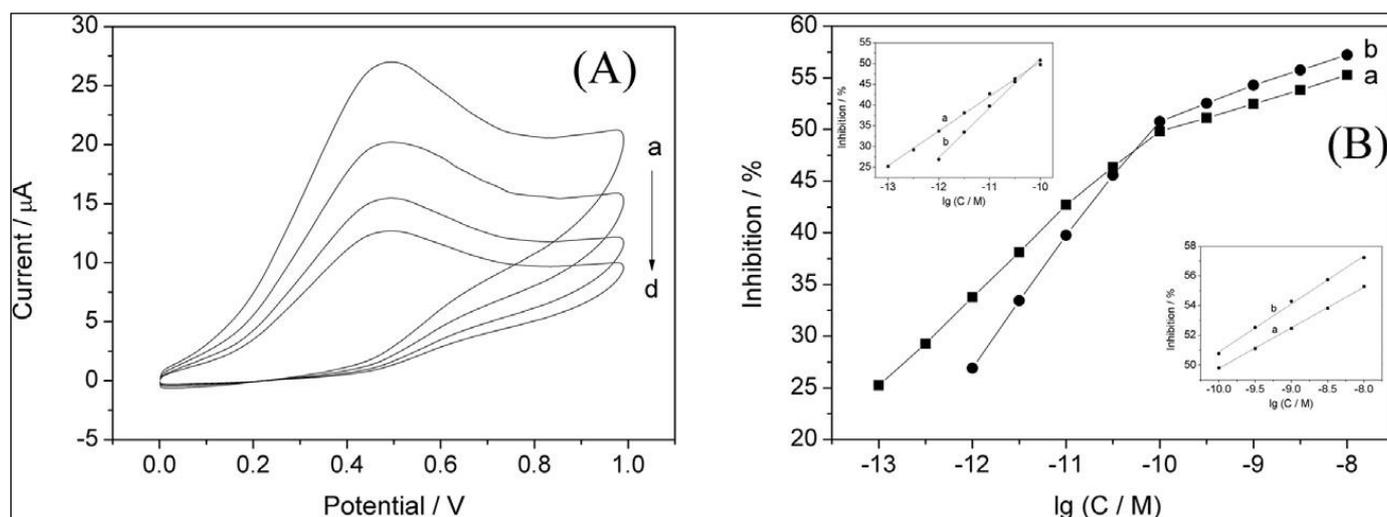


Fig 1: (A) CV of the NF/AChE-CS/PtNPs-CGR-NF/GCE in pH 7.4 PBS containing 0.5 mmolL^{-1} ATCI after incubation with 0 (a) 10^{-13} molL^{-1} (b) 10^{-11} molL^{-1} (c) 10^{-9} molL^{-1} (d) methyl parathion for 6 min. (B) Inhibition curves of NF/AChE-CS/PtNPs-CGR-NF/GCE biosensor for methyl parathion (a) and carbofuran (b) determination after 6-min incubation [37]

GCE was modified with SnO_2 NPs, CGR and NF, a highly sensitive and stable AChE biosensor for detection of carbofuran and methyl parathion has been developed. CGR nano composites and SnO_2 NPs were characterised by XRD, SEM and FTIR methods. AChE was immobilized by CS on SnO_2 NPs-CGR-NF/GCE having biocompatibility and

excellent conductivity. AChE shows affinity with ATCI and catalyze ATCI hydrolysis with Michaelis-Menten constant (K_m) value of $13 \mu\text{molL}^{-1}$. Linearity range of methyl parathion was from 10^{-13} to 10^{-8} molL^{-1} . Linearity range of carbofuran was from 10^{-12} to 10^{-8} molL^{-1} . The detection limits of carbofuran and methyl parathion were 5.0×10^{-13} molL^{-1} and

$5.0 \times 10^{-14} \text{ molL}^{-1}$ respectively [38]. MWCNT due to large ratio of porosity and good electrical conductivity provide a larger pathway and flexible conductive film, due to these features sensitivity of detector increases tremendously. Redox process is promoted by SnO_2 materials as peak voltage was found to be decrease by applying SnO_2 film. MWCNTs- SnO_2 -CS have excellent conductive properties, so AChE had high affinity for ATCI which catalyse hydrolysis of ATCI. AChE biosensor formed have good selectivity, high stability and sensitivity and gives excellent response for chlorpyrifos detection having detection limit $0.05 \mu\text{gL}^{-1}$ and linearity range 0.05 to $1.0 \times 10^3 \mu\text{gL}^{-1}$ [39].

Zhang *et al.* and Huang *et al.* modified GCE surfaces by using AuNPs and PdNPs combined with MWCNTs. The GCE surface was modified with $5 \mu\text{L}$ of a 0.5% m/m Nafion aqueous dispersion containing CNTs. Using CV the AuNPs were electrodeposited on the CNTs/GCE (between -0.2 V and +1.0 V vs. SCE) in $0.2 \text{ molL}^{-1} \text{ H}_2\text{SO}_4$ solution containing $5.0 \text{ mmolL}^{-1} \text{ HAuCl}_4$. The authors showed that AuNPs possess electrocatalytic activity towards the reduction of parathion. The curve obtained using LSV was linear from 5.0×10^{-7} to $6.0 \times 10^{-5} \text{ molL}^{-1}$, with a detection limit of $1.0 \times 10^{-7} \text{ molL}^{-1}$. The developed voltammetric procedure was applied in the parathion quantification of spiked water and vegetable samples with recovery percentages ranging from 98.3% to 104.3% [40].

Polymer based electrodes

For analysis of OPs, conducting polymers like polypyrrole (PPy), polyaniline (PAn), polyindole, poly acetylene, polythiophene, polyfuran, poly (para-phenylene), poly(o-phenylenediamine) and poly(3-hydroxythiophene), are generally used as electrode coating materials in electrochemical sensing method [41, 42]. AChE was immobilized on copolymer of polyaniline and polypyrrole doped with MWCNTs. Homogeneous and porous morphology of PAn PPy-MWCNTs copolymer provides an ideal size for trapping enzyme molecules. The copolymer network provide biocompatible microenvironment, due to this the composite was devised for AChE attachment, resulting in a stable AChE biosensor for OP screening. MWCNTs promoted electron-transfer reactions at a lower potential and catalyzed the electro-oxidation of thiocholine, thus increasing detection sensitivity of working electrode. Based on the inhibition of OPs on the AChE activity, using malathion as a model compound, the inhibition of malathion was proportional to its concentration ranging from 0.01 to 0.5 gmL^{-1} and from 1.0 to 25.0 gmL^{-1} , with a detection limit of 1.0 ngmL^{-1} (Fig. 2). The developed biosensor exhibited good reproducibility and acceptable stability, thus providing a new promising tool for analysis of enzyme inhibitors [43].

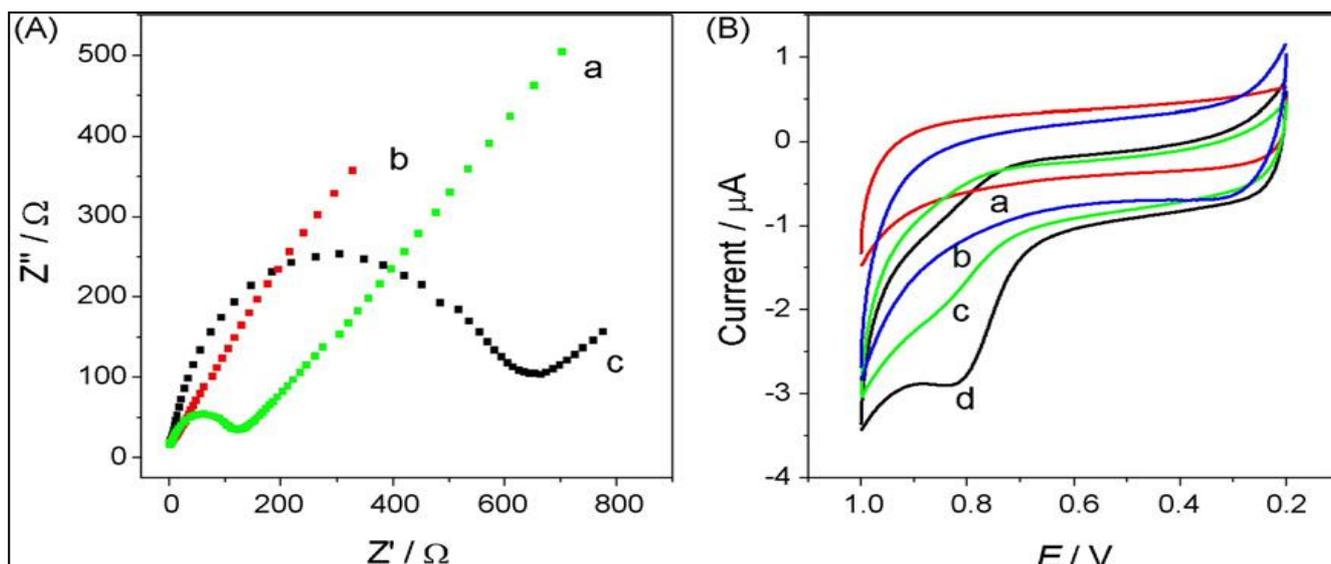


Fig 2: (A) Electrochemical impedance spectra of bare GCE (a), PAn-PPy-MWCNTs/GCE (b) and AChE-PAn-PPy-MWCNTs/GCE (c) in 5mM $\text{K}_3\text{Fe}(\text{CN})_6$ and $\text{K}_4\text{Fe}(\text{CN})_6$. (B) Cyclic voltammograms of bare GCE (a) and AChE-PAn-PPy-MWCNTs/GCE (b) in pH 7.0 PBS; AChE-PAn-PPy/GCE without MWCNTs (c) and AChE-PAn-PPy-MWCNTs/GCE (d) in pH 7.0 PBS containing $0.3 \text{ mmolL}^{-1} \text{ ATCI}$. Scan rate: 50 mVs^{-1} [46]

Li *et al.* and Sundari *et al.* modified GCE surface with CNTs trapped into electrochemically prepared polymeric matrices, determine carbendazim using LSV by designing GCE modified with MWCNT-polymeric methyl red film; a detection limit of 9.0 nmolL^{-1} and satisfactory recoveries for water samples were achieved (between 90.3% and 94.7%) [44]. Sundari *et al.* use GCE modified with MWCNTs and poly(3-methyl thiophene) film, which is a conducting polymer and EC response of various pesticides (cypermethrin, deltamethrin, dicofol, fenvalerate isoprotruron, and voltage) were evaluated [45].

Inam *et al.* detected the methiocarb insecticide by SWV using

a MWCNT paste electrode. CNT powder based CNPE was prepared by mixing mineral oil with CNT powder in the 0.15:0.85 w/w ratio. An irreversible anodic peak at +1.3 V vs. Ag/AgCl ($3.0 \text{ molL}^{-1} \text{ NaCl}$) was obtained when methiocarb in $0.1 \text{ molL}^{-1} \text{ H}_2\text{SO}_4$ was electrochemically characterized, which was used for its quantification. LDR was between 6.7×10^{-6} and $2.6 \times 10^{-4} \text{ molL}^{-1}$ and a detection limit of $2.0 \times 10^{-6} \text{ molL}^{-1}$. In river water samples and soil the sensor was applied for determining methiocarb and recovery was (98.5-0.3) %, (99-1) % respectively. An increase in the recorded analytical signals was seen when CNT based sensors were combined with SWSV [46].

Table 1: Nanoparticle based fabrication of OP biosensors

| S. No. | Target analyte | Detection technique | Immobilization technique | Electrode | Primary coating | Secondary coating | Tertiary coating | Linearity Range | L.O.D | References |
|--------|--------------------------------|---------------------|--------------------------|--------------------------|--------------------|--------------------------------|------------------|---|---|------------|
| 1. | Orthonitro phenol | CV | NA | GCE | PPI dendrimer | AuNP | | | 4.5×10^{-7} | [50] |
| 2. | Chlorpyrifos | CV | | Screen printed electrode | MWCNT and Chitosan | SnO ₂ | AChE | $0.05-1 \times 10^3 \mu\text{gL}^{-1}$ | $0.05 \mu\text{gL}^{-1}$ | [34] |
| 3. | Paraoxon | Amperometry | Adsorption | | AuNPs | Graphene Oxide nano sheets | | ND | 10^{-13} | [51] |
| 4. | Monocrotophos | Amperometry | Covalent binding | | AuNPs | QDS | | $4.5 \times 10^{-9}-4.5 \times 10^{-6}$ | 1.3×10^{-9} | [52] |
| 5. | Methyl Parathion and Acephate | Amperometry | Encapsulation | CPE | Silicasol-gel film | | | $3.7 \times 10^{-4}-1.8 \times 10^3$ | $3 \times 10^{-4} \mu\text{molL}^{-1}$ | [53] |
| 6. | Dimethylvinphos and Naftalofos | SWV and CV | | GCE | Chitosan | REDUCED graphene oxide | | $0.50-30 \mu\text{gL}^{-1}$ | For DMV- 0.036mgL^{-1} For Naftalotos- 0.044mgL^{-1} | [54] |
| 7. | Dichlorvos | DPV | Adsorption | SPE | Sol gel matrix | Al ₂ O ₃ | | $0.1-80 \mu\text{M}$ | $0.01 \mu\text{M}$ | [55] |
| 8. | Paraoxon | Amperometric | | SPE | Sol gel | Zinc oxide | | $0.127-5.010 \mu\text{M}$ | $0.127 \mu\text{M}$ | [56] |
| 9. | Monocrotophos | Electrochemical | Hydrogen bonds | GCE | SiSG | AuNPs | | NR | $0.44 \mu\text{M}$ | [57] |
| 10. | Chlorpyrifos-ethyl oxon | Amperometric | Encapsulation | Carbon electrode | | Alumina | | $0.5 \mu\text{M}$ | $2.5 \times 10^{-6} \mu\text{M}$ | [58] |

Conclusion

The review presented clears that the biosensor for OP detection have various applications in the estimation of pesticides in environment. NPs are proved to be suitable for fabrication of WE due to their high conductivity, efficient electron transfer mechanisms and can be prepared by simple techniques. NPs also increase the self-life of AChE biosensors. A wide range of WE have been prepared by surface modification which includes electrodes based on metals, metal oxides, polymers and their composites. These designed sensors provide a sensitive and selective method for the quantification of OP pesticides thus help in reducing environmental losses and improving human health.

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