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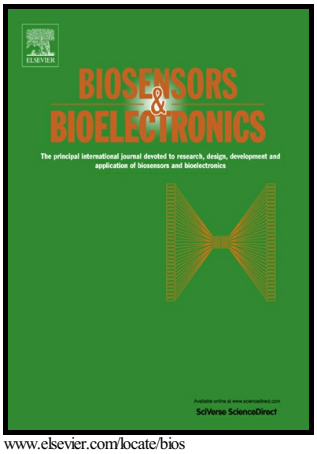
Shivani Uniyal, Rajesh Kumar Sharma



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Technological advancement in electrochemical biosensor based detection of Organophosphate pesticide chlorpyrifos in the environment: A review of status and prospects

Shivani Uniyal, Rajesh Kumar Sharma*

Department of Botany, Banaras Hindu University, Varanasi-221005, India

*Corresponding author. Tel.: 91-9418916445. Email: rajeshbhu78@gmail.com

Abstract

Chlorpyrifos (CP), an organophosphate insecticide is broadly used in the agricultural and industrial sectors to control a broad-spectrum of insects of economically important crops. CP detection has been gaining prominence due to its widespread contamination in different environmental matrices, high acute toxicity, and potential to cause long-term environmental and ecological damage even at trace levels. Traditional chromatographic methods for CP detection are complex and require sample preparation and

highly skilled personnel for their operation. Over the past decades, electrochemical biosensors have emerged as a promising technology for CP detection as these circumvent deficiencies associated with classical chromatographic techniques. The advantageous features such as appreciable detection limit, miniaturization, sensitivity, low-cost and onsite detection potential are the propulsive force towards sustainable growth of electrochemical biosensing platforms. Recent development in enzyme immobilization methods, novel surface modifications, nanotechnology and fabrication techniques signify a foremost possibility for the design of electrochemical biosensing platforms with improved sensitivity and selectivity. The prime objective of this review is to accentuate the recent advances in the design of biosensing platforms based on diverse biomolecules and biomimetic molecules with unique properties, which would potentially fascinate their applicability for detection of CP residues in real samples. The review also covers the sensing principle of the prime biomolecule and biomimetic molecule based electrochemical biosensors along with their analytical performance, advantages and shortcomings. Present challenges and future outlooks in the field of electrochemical biosensors based CP detection are also discussed. This deep analysis of electrochemical biosensors will provide research directions for further approaching towards commercial development of the broad range of organophosphorus compounds.

Keywords: Chlorpyrifos · Electrochemical Biosensors · Nanotechnology · Fabrication techniques · Biomimetic

1. Introduction

The organophosphate pesticides represent one of the most frequently used class of pesticides in agricultural practice for more than 40 years (Cycon et al. 2009). Chlorpyrifos, O,O-diethyl O-(3,5,6-trichloro-2-pyridyl), is a broad spectrum organophosphate insecticide used world-wide to restraint a wide range of insects and pests on crops, including cereals, cotton, vegetables, nuts and grains (Wang et al., 2007). CP was reported as the fourteenth most often used conventional active ingredient with 3.50 -4.50 thousand tons use in the market sector for agricultural pesticides in the year 2007 (Grube et al., 2011). Residential use of CP ceased in the United States in the year 2001 due mainly to safety concerns linked with greater neurotoxic effects in children as compared to adults (USEPA, 2000; USEPA, 2006). However, it has still been used in the range of 3.2–4.1 thousand tons year^{-1} since 2008, proving primary route of CP exposure via dietary residues (USEPA, 2011; Solomon et al., 2014). CP is lipophilic in nature with a log P value of 4.96 (Sangster, 1994). CP exerts its toxicity primarily by inhibiting acetylcholinesterase (AChE) activity and thus affecting central and peripheral cholinergic nervous systems (Mileson et al., 1998). CP can bind irreversibly to the active site of AchE, thus inhibiting the hydrolysis of the neurotransmitter acetylcholine (ACh), resulting in its accumulation at the neuronal cholinergic synapses (Howard et al., 2007). This could affect cholinergic signaling activity of the enzyme, triggering cell death in cholinergic neurons in the basal forebrain and can induce increased CHT expression and alteration in acetylcholine levels, after long-term exposure (Howard et al., 2007; Del Pino et al., 2016). However, it can undergo biotransformation to oxygen (oxon) analogue i.e. chlorpyrifos oxon (CP-O) by a cytochrome P450 monooxygenase system in liver (Sultatos et al., 1984). CP-O, thus formed is reported to inhibit AChE activity with 180 times more potency than CP itself in differentiated brain cells (Monnet-Tschudi et al., 2000). Fig.1. represents the chemical structure of CP and CP-oxon.

The overuse of CP and their improper storage or disposal is a major input of potential environmental contamination. The detection of its environmental levels is therefore essential to minimize the ecological and health risks associated with CP exposure. Thus, extensive researches have been directed to develop fast and reliable techniques for CP detection. Official methods for CP detection usually involve sophisticated instrumentation like gas chromatography (GC), high pressure liquid chromatography (HPLC), and mass spectrometry (MS). However, these complicated, expensive and time consuming detection methods encouraged the demand for small devices for rapid *insitu* CP detection. Electrochemical biosensors have transpired as an attractive alternative strategy since these are sensitive, simple and apt for online and onsite measurements. Moreover, since electrochemical biosensors are based

on the generation of an electronic signal by electrochemical reactions involved in the electron transfer process, these potentially avoid the prerequisite for expensive equipment (Belluzo et al., 2008). In the last couple of years, numerous electrochemical sensing platforms have been designed by the conjunction of functional nanomaterials, surface modification of electrodes and microfabrication technology to intensively pursue CP detection. Further, vast endeavors have been dedicated to investigating new sensing probe materials to improve the detection capability and biocompatibility of these sensors (Moon et al. 2018). The present review focuses on the recent advances in the growth of various biomolecules and biomimetic molecules based electrochemical biosensors for CP detection. Additionally, evolutionary progress in the development of novel immobilization methods and functional materials used to design biosensing platforms with improved performances are also addressed. Beyond a discussion of the efficient fabrication methods for the rational configuration of electrochemical biosensors, we also highlighted prevailing challenges and future outlooks that might influence commercialization opportunities.

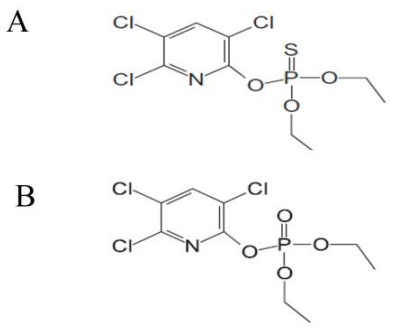

Fig.1. Chemical structures of (A) Chlorpyrifos (B) Chlorpyrifos-oxon

2. Status of CP contamination: A global scenario

Meanwhile, several regions around the world contaminated with CP have been discovered particularly through surveying the occurrence of CP in various environmental matrices. Recent water quality monitoring studies in California indicated exceeded concentrations of CP residues (Phillips et al., 2007; Corbin et al., 2009; Ensminger et al., 2011). Similarly, CP was detected at a high concentration i.e. 3.7 µg L^{-1} in San Joaquin region (Xuyang et al., 2012). In another study, data from National Water-Quality Assessment (NAWQA) and National Stream Quality Accounting Network (NASQAN) was used to generate estimates for CP concentration (Mosquin et al., 2015). Results indicated that upper bound estimates for CP ranged between <0.005 to 0.0852 µg L^{-1}. CP residues were also detected in indoor air at concentrations of 6 mg m^{-3} and 0.7 mg m^{-3}, while in the soil, it was 499 mg kg^{-1} and 295 mg kg^{-1} after 4 and 8 years of application, respectively (Wright et al., 1994). Pozo et al. (2016) studied seasonal variation in the atmospheric level of CP in the Araucania Region, a central-southern area of Chile. The concentration of CP in the Chilean atmosphere was ranged from ten to thousands of pg m^{-3}(~20-14600). CP was also detected with values ranging 3-580 pg m^{-3} and 3-430 pg m^{-3} at urban and rural sites respectively, in the Region of Tuscany, Italy (Estellano et al., 2015). Atmospheric concentrations of CP up to 1428 ng m^{-3} have been reported in both the gas and particle phases (Yusa et al., 2009; Borras et al., 2011). Lehotay et al. (1998) monitored CP concentration in oysters and water in the Chesapeake Bay of USA and detected low CP concentrations after the agricultural season. Sun and Chen (2008) studied one hundred thirty-seven farmed and wild fish samples for the presence of CP residues and observed about 23% CP detection rate in farmed fish, which was quite higher than wild fish.

Table 1 An Overview of environmental regulations for chlorpyrifos

S.No.	Environmental quality standards	Commodity	Regulatory criterion	Regulatory values	Reference

1	European standards	Inland surface water	Maximum allowable concentration (MAC)	0.1 µg L^{-1}	EU, 2015
		Other surface water	Maximum allowable concentration (MAC)	0.1 µg L^{-1}	
2	USEPA	Freshwater	Water quality criterion	Water quality criterion Acute 0.07 µg L^{-1} implemented on a one hour average and chronic 0.041 µg L^{-1} implemented on a four day average	US EPA, 1987
		Salt water	Water quality criterion	Acute 0.056 µg L^{-1} and chronic 0.03 µg L^{-1}	
3	WHO	Drinking water	Acceptable daily intake (ADI)	30 µg L^{-1}	WHO, 2004
4	CPCB	Effluent	Limiting concentration	100 µg L^{-1}	MOEF & CC, 2011
5	Electronic code of federal regulations	Apple	Tolerances for residues	0.01 µg g^{-1}	US GPO, 1996
		Pumpkin	Tolerances for residues	0.05 µg g^{-1}	
		Corn, field, grain	Tolerances for residues	0.05 µg g^{-1}	

3. Environmental risk implications of CP

Neonatal CP exposure was observed to interfere with synaptic proliferation and processes in the midbrain and striatum (Dam et al., 1999; Slotkin et al., 2002). It can also disrupt the functioning of the cerebellum, the region of the brain that is associated with the control of movement, thus causing tremor (Slotkin and Seidler, 2005). It can induce oxidative stress, increase in H_2O_2 and malondialdehyde content and reduction in cell viability through upregulation of P75NTR and downregulation of a7-nAChRs expression (Del Pino et al., 2016). CP exposure can disrupt reproductive functionality by affecting morphologically sperm motility, reducing sperm count, production of oviposition and increasing sperm DNA fragmentation and instances of dead fetuses (Farag et al., 2010; Meeker et al., 2004). Several studies had indicated that environmentally realistic concentration of CP is reported to induce significant toxic effects to living organisms. For instance; prenatal exposure of CP was reported to cause a significant reduction in body weight and brain volume of the offspring in the guinea pig (Mullins et al., 2015). Results indicated that anterior forebrain suffered most damage with major effect on striatum, amygdala, and corpus callosum, thus affecting cognitive processing. Similar results were observed for rats, in which prenatal CP exposure affected neural development and triggered neurocognitive disorders later in life

(Levin et al., 2001, 2002; Turgeman et al., 2011). In addition, CP exposed rat showed a significant diminution in testosterone biosynthesis, antioxidant enzyme levels, increase in the level of lipid peroxides in different organs, and disturbance in an antioxidant system in the liver, kidney, brain, and fetus (Viswanath et al., 2010; Zama et al., 2007). Moreover, repeated exposure to CP could affect endocannabinoid signaling in juvenile rat brain by inhibiting a endocannabinoid anandamide hydrolyzing enzyme i.e. fatty acid amide hydrolase (FAAH), which result into increase in anandamide accumulation (Carr et al., 2014). In mice, it can induce impairments in attention and motivation, reduction in anticipatory responding, delayed attentional disruptions (Peris-Sampedro et al., 2016) and embryo toxicity even at lower doses than those known to be associated with significant maternal toxicity (Tian et al., 2005). CP exposure in dainty bluet, a Mediterranean damselfly was also studied (Dinh et al., 2016). Results indicated frequent incidences of wing malformations during metamorphosis, a lower mass at emergence in females, a lower relative flight muscle mass in edge males and affected adult immune response. In zebrafish embryo, CP is reported to alter the mRNA expression levels of c-myc, cyclin D1, Bax and Bcl-2, thereby disturbing cell proliferation, embryonic hatching, and cell apoptosis (Yu et al., 2015). In a similar study, CP exposure in rainbow trout was observed to affect olfactory signal transduction by interacting with protein kinase II delta, G protein zgc:101761 and adrenergic alpha 2C receptor (Sandahl et al., 2004). In humans, CP exposure is reported to induce attention deficit/hyperactivity disorder (ADHD) and alteration in the brain morphology in children (Rauh et al., 2006; Rauh et al., 2012).

4. Analytical techniques for CP Detection

Currently, the methods of CP detection usually involve gas chromatography (GC), high pressure liquid chromatography (HPLC), mass spectrometry (MS) and are preferred due to their efficiency, sensitivity, and reliability; however, these conventional methods are quite expensive, time consuming, require trained personnel, sample pretreatment, and are not suitable for real-time detection (Fenoll et al., 2009; Pinxteren et al., 2009; Lesueur et al., 2008 (a, b); Filho et al., 2010). Bioanalytical techniques comprising the use of biosensors have progressed to evolve as a promising and revolutionary strategy for rapid screening of CP. A biosensor can be used for quantitative determination of CP in different environmental samples. As biotechnology-based methods for biosensor fabrication advances rapidly, several kinds of biosensors have been developed, inventing novel approaches for amplifying detection performance. A biosensor employs the use of an immobilized biological element (e.g., nucleic acids, proteins, metabolites, isoenzymes, hormones, enzyme or antibody) for the recognition of the analyte (e.g., enzyme, substrate or antigen) to generate a biochemical signal (Zhang et al., 2014). These signals are converted into electronic or optical signals by signal transducing element (electrode, optical detector, piezo crystal), which are amplified and detected by an electronic unit (Songa et al., 2009). Several kinds of biosensors being used are the enzyme, immunosensors, DNA, electrochemical, optical, piezoelectric, thermal, and magnetic biosensors (Wang et al., 2014). Among all the biosensors, electrochemical sensors are reported to be used in major for environmental monitoring of CP and are the main subject of this review.

5. Electrochemical Biosensors: A Trend toward CP Detection

Electrochemical biosensors for CP detection are prominent due to their marked characteristics to provide sensitivity, specificity, and reproducibility. They exhibit either two electrode system consists of working and reference electrodes or three electrode system consisting of an additional auxiliary electrode (Moon et al., 2018). These biosensors are based on the selective interaction between the target compound and recognition element to engender an electrical signal that is related to the concentration of the compound being studied. They can work on different detection principle including amperometry, potentiometry, conductometry, voltammetry and electrochemical impedance spectroscopy (EIS). Amperometry estimates the current engendered as a result of constant reducing or oxidizing potential applied to the working electrode. The resultant current intensity is used for quantification of reduced or

oxidized species. In potentiometry, a potential difference between the working electrode and a reference electrode, induced by changes in the concentration of charged species, is measured. In conductometry, conductance caused by the mobility of ions towards cathode and anode is measured. In voltammetry concentration of an electroactive species is determined by amperometric measurement at an electrode as a function of a voltage applied to the electrode. EIS evaluates the resistances and capacitances by applying small amplitude sinusoidal AC excitation signal at the given frequency range. Resulting EIS plots indicating the magnitude of the impedance vector of a particular frequency can be further used for quantification of analyte.

Table 2: Electrochemical biosensors for chlorpyrifos detection

Electrode configuration	**Detection method**	**Detection limit**	**References**
Graphite/TCNQ/HEC/sol-gel/AChE*	Amperometry	4.4×10^{-4} µg mL^{-1}	Andreescu et al., 2002
dsCT-DNA-PPyPVS/ITO	Voltammetry	1.6×10^{-3} µg mL^{-1}	Prabhakar et al., 2007
dsCT-DNA entrapped PANIPVS/ITO	Voltammetry	5×10^{-4} µg mL^{-1}	Prabhakar et al., 2008
AChE/Al_2O_3/Sonogel-carbon*	Amperometry	8.8×10^{-5} µg mL^{-1}	Zejli et al., 2008
Au/ssDNA– SWCNT/PANI/ AChE	Voltammetry	3.5×10^{-7} µg mL^{-1}	Viswanathan et al., 2009
PEDOT: PSS/SPE*	Amperometry	1.4×10^{-3} µg mL^{-1}	Istamboulie et al., 2010
AChE–chitosan /GCE	Voltammetry	5.6×10^{-3} µg mL^{-1}	Ion et al., 2010
AChEPin5COOH/ZnS/AuE	Amperometry	5.3×10^{-4} µg mL^{-1}	Chauhan et al., 2011
CPBA/AuNPs/RGO-CS/GCE	Amperometry	1×10^{-4} µg mL^{-1}	Liu et al., 2011a
Cysteamine/ Nano-Pt/Graphene/GCE	Amperometry	2×10^{-4} µg mL^{-1}	Liu et al., 2011b
AChE/Fe_3O_4/c-MWCNT/Au	Amperometry	3.50×10^{-5} µg mL^{-1}	Chauhan and Pundir, 2011
[BMIM][BF4]/MWCNT-CP	Amperometry	1.30×10^{-3} µg mL^{-1}	Zamfir et al., 2011
Fe_3O_4NPs/c-MWCNTs/ITO	Voltammetry	3.50×10^{-5} µg mL^{-1}	Chauhan and Pundir, 2012
BSA/antichlorpyrifos/AuNPs/PANI/MWCNTs/ CHIT/ GCE	Voltammetry	6×10^{-5} µg mL^{-1}	Sun et al., 2013
AChE–CS/SNS–NF/GCE	Amperometry	1.45×10^{-5} µg mL^{-1}	Yang et al., 2013
AChE/Chit–PB–MWNTs–HGNs/Au	Amperometry	1.75×10^{-5} µg mL^{-1}	Zhai et al., 2013
AChE/AuNPs-CSs/BDD	Voltammetry	4.55×10^{-8} µg mL^{-1}	Wei et al., 2014
CLDH-AChE/GN-AuNPs/GCE	Voltammetry	50 µg mL^{-1}	Zhai et al., 2014
MIP/PBS/TPA	Potentiometry	1.08×10^{-5} µg mL^{-1}	Li et al., 2015
Fc@MWCNTs/OMC/GCE	Voltammetry	3.33×10^{-4} µg mL^{-1}	Jiao et al., 2016
AChE/ZrO_2/RGO	Voltammetry	3.50×10^{-8} µg mL^{-1}	Mogha et al., 2016
Fe_3O_4NPs/Pin5COOH	Voltammetry	5.25×10^{-5} µg mL^{-1}	Chauhan et al., 2016
CuONFs-	Voltammetry	7×10^{-5} µg mL^{-1}	Xu et al., 2017

SWCNTs/Nafion/GCE			
NA/Ag@rGO-NH_2/ AChE/GCE	Voltammetry	1.4×10^{-2} µg mL^{-1}	Guler et al., 2017
MIP-B-TiO_2 NRs	Voltammetry	7.4×10^{-6} µg mL^{-1}	Sun et al., 2017
FTO-AuNPs	Voltammetry	3.50×10^{-3} µg mL^{-1}	Talan et al., 2018

*Analyte is CP-oxon.

Table 2 summarizes the recently reported electrochemical biosensors in the literature. The following sections feature some of the important electrochemical biosensors that have been implemented for CP detection.

5.1 Biomolecules based electrochemical biosensors

The field of biomolecules based electrochemical biosensors has experienced substantial progress, aimed principally towards the exploitation of different kinds of recognition elements for achieving simplicity, reliability and high sensitivity. These biosensors can be discriminated into four system formats: DNA, immunosensors, aptasensors and enzymatic biosensors.

5.1.1. DNA biosensors

DNA biosensors use DNA as a bio-recognition element, attached to the surface of the working electrode to generate an electrochemical response. An organophosphorus compound like CP bearing phosphorothioate group may damage the entrapped DNA by phosphorylating the amino group at C-2 position of guanine base (Rahman et al., 2002). It may result to an inaccessible lone pair of the amino group at C-2 position of guanine for oxidation (Prabhakar et al., 2007). Thus, these bulkier groups of pesticides result to the decrease in guanine oxidation current by resisting the electron flow (Prabhakar et al., 2007). Further, these changes in the DNA redox properties (i.e. oxidation of guanine) are used to the target analyte. DNA biosensors can also function by intercalation of an electro-active analyte on a DNA layer. These sensors offer advantages as better compatibility with micro-fabrication technology and can be constructed with a broad spectrum of conducting/semi-conducting matrices such as gold, platinum, conducting polymers, etc. (Prabhakar et al., 2008; Wong et al., 2006). In a study, polypyrrole-polyvinyl sulphonate (dsCT-DNA-PPy-PVS) coatings, entrapped with *calf thymus* deoxyribonucleic acid were prepared and assembled onto indium-tin-oxide (ITO) (Prabhakar et al., 2007). The obtained dsCTDNA-PPy-PVS/ITO bioelectrodes showed high performance for CP detection. CP detection strategy was based on the change in voltammetric patterns of the modified electrode due to oxidation of guanine. The fabricated biosensor exhibited the detection limit of 1.6×10^{-3} µg mL^{-1} within the 30s. Presence of inorganic salts exhibited no inhibitory effect on biosensor signal. Following this work, Prabhakar et al. (2008), further developed a novel bioelectrode system comprising dsCT-DNA entrapped polyaniline (PANI)-polyvinyl sulphonate (PVS) films, fabricated onto indium-tin-oxide (ITO). Amperometric response of the constructed biosensor was not altered by the presence of inorganic salts. Change in voltammetric characteristics of the modified electrodes has been used to recognize CPs within the 30 s of response time with a detection limit of 5×10^{-4} µg mL^{-1}. DNA based biosensors displayed robust electrochemical activity and stability for CP detection. However, issues about selectivity and reproducibility are some of the bottlenecks needed attention.

5.1.2. Immunosensors

The introduction of antibodies or antigens as the specific recognizing unit further extends the applications of a bio-molecule based biosensor. They offer advantages like portability, the requirement of low sample volume and have an eminent potential for automation (Afonso, 2012). In addition, high

selectivity in view of immunological interactions provides more opportunity for specific recognition. In immunosensors, antibodies act as a specific recognizing element and generate signals which are correlated with the concentration of an analyte. An electrochemical immunosensor was fabricated for detection of CP (Cao et al., 2015). In this sensor, immobilization of anti-chlorpyrifos monoclonal antibodies was performed onto the gold microelectrode surface. Target CP molecules were grasped by the immobilized antibody onto the electrode surface, which substantially increased impedance compared with a control sample. In this study, the electrochemical impedance spectroscopy (EIS) technique was applied to study the redox reaction of the fabricated IDAMs. These impedance changes in the fabricated biosensor were used for CP detection. Results confirmed CP detection with a detection limit of 1.4×10^{-5} µg mL^{-1}. The proposed immunosensor exhibited an extortionate selectivity for CP detection in the presence of other non-target pesticides. In another study, fluorine doped tin-oxide (FTO) based biosensor was used for CP detection with gold nanoparticles and anti-chlorpyrifos antibodies (chl-Ab) (Talan et al., 2018). CP was detected by monitoring the changes in cyclic voltammetric patterns. The proposed biosensor exhibited highly sensitive CP detection, with detection limit up to 3.50×10^{-3} µg mL^{-1}. However, the low chemical/physical stability limits the application of antibodies in harsh environments such as acids, organic solvents, and high temperature (Li et al., 2012).

5.1.3. Aptasensors

Compared with the antibody, the aptamer has apparent advantages of accurate chemical recognition, ease of modification, *in vitro* synthesis, high purity and thermal stability (Jayasena, 1999; Jiao et al., 2016). Aptamers are single stranded oligonucleotides or peptide molecules that are capable of recognizing and binding a variety of targets (Song et al., 2012). These can undergo partial hybridization with capture probe on the electrode surface (Dai et al., 2012). The hybridization process can be monitored by application of a redox indicator, which can bind with nucleic acid chains and specifically recognize unbound guanine base (Erdem et al. 2001; Gorodetsky et al. 2008). However, the amount of redox indicator bound to guanine bases in the aptamer domain may be reduced in the presence of analyte attributing to the formation of more compact aptamer-CP complex structure (Xu et al., 2017). It may cause aptamer dissociation from semi-duplex, release of redox indicator from the sensing interface and consequent decrease of peak current with increasing analyte concentration (Xu et al., 2017). These changes in the peak current intensity can be used for quantification of the target analyte. Aptamer based biosensors have earned recognition in the field of CP detection. In a study, a regenerative electrochemical aptasensor was constructed for the sensitive detection of CP (Xu et al., 2017). The biosensor was fabricated by incorporating CuO nanoflowers (NFs) and carboxyl-functionalized single-walled carbon nanotubes (c-SWCNTs). Selectivity of the propounded biosensor was evaluated in the presence of other interfering pesticides and results indicated a good interference resistance, confirming remarkable selectivity. Changes in the differential pulse voltammetric patterns of the fabricated biosensor were used to detect CP. The sensor showed a low detection limit of 7×10^{-5} µg mL^{-1}. Similarly, an ultrasensitive aptasensor was designed for CP detection by amalgamating the special features of mesoporous carbon (OMC), chitosan (OMC-CS) ferrocene hybrid chitosan (CS) and multiwalled carbon nanotubes (MWCNTs) (Fig. 2A) (Jiao et al., 2016). Cyclic voltammograms of different modified electrodes revealed that the conductance could be promoted by Fc@WMCNTs-CS and partially restricted by the aptamer and BSA (Fig. 2B). Selectivity study indicated no amperometric alteration in the presence of interfering molecules confirming selective recognition ability of the aptasensor (Fig. 2C). CP was detected and quantified by evaluating the changes in cyclic voltammetric characteristics of the modified aptasensor. The proposed aptasensor demonstrated remarkable selectivity for CP and exhibited a low detection limit of 3.33×10^{-4} µg mL^{-1}. Despite the high sensitivity for CP detection, aptamer-based biosensors still require more exploration of immobilization strategies to increase the ease of biosensor fabrication.

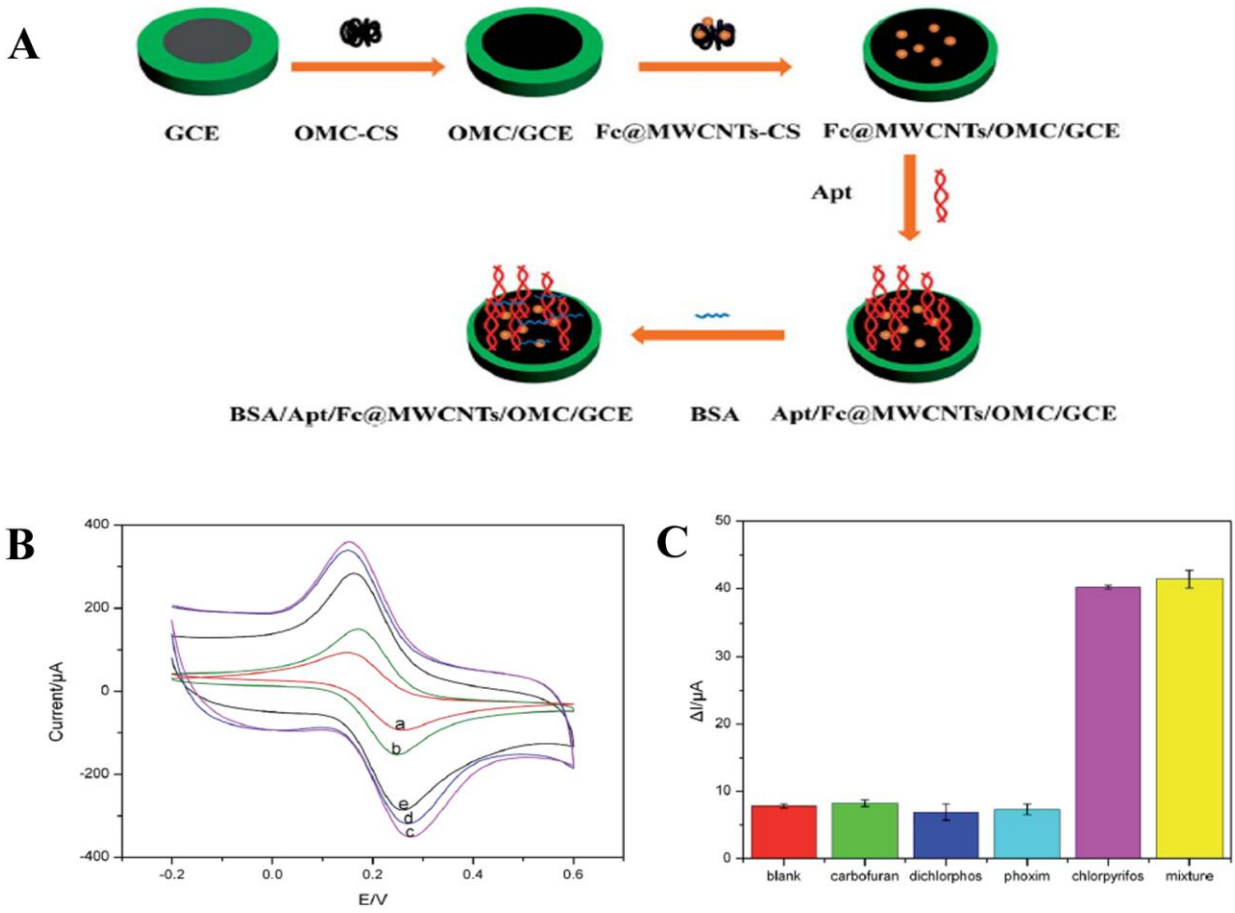

Fig. 2. (A) Stepwise preparation of the aptasensor. (B) Cyclic voltammograms of (a) bare GCE (b) OMC/GCE (c) Fc@MWCNTs/OMC/GCE (d) Apt/Fc@MWCNTs/OMC/GCE (e) SA/Apt/Fc@MWCNTs/OMC/GCE. (C) Selective detection of CP against the interference molecules, carbofuran, dichlorphos, phoxim, mixture of the interference molecules and CP. Reproduced with permission from Ref. Jiao et al., 2016, Copyright 2016 Royal society of chemistry.

5.1.4. Enzyme based sensors

Enzyme based inhibition system hold great promise for confronting the analytical requirements in Organophosphate pesticide detection. In enzyme based sensors, an enzyme is immobilized on the surface of the working electrode by physical or chemical processes. Recently, AChE enzyme based biosensors have gained attention in the field of CP detection. The active catalytic centre of AChE comprised of two sites viz. cationic and anionic (Hematpoor et al., 2016), which electrostatically binds with the carbonyl group and ammonium group of Acetylthiocholine (ATCl), respectively (Viswanathan et al., 2009). The serine hydroxyl group of AChE cleaves the ATCl and consequently releases thiocholine and two electrons in the process, indicating electro-activity of the electrode (Mogha et al., 2016). However, an amperometric signal is reduced in the presence of CP, as it undergoes dealkylation by covalently binding to the cationic site via phosphate group, resulting in irreversible binding to AChE (Antoni et al., 2005). This decrease in the sensor response can be co-related to CP concentration.

Various enzyme based biosensors have been reported with slight modifications in immobilization method to ensure sensitive CP detection such as self-assembled monolayers based, gel based, polymer based, carbon nanotube based, graphene based, metal and metal oxide based biosensors.

5.1.4.1. Carbon nanotubes (CNT) based biosensors

Carbon nanotubes are hollow graphitic cylinders with the variable number of rolled layers of nested graphene sheets (Viswanathan et al., 2008). CNTs with a single layer and multiple layers of graphene sheet are characterized as single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes, respectively. Though both kinds are known as strong candidates for fabrication of electrodes due to their strong electrocatalytic effect, MWNTs are widely applied owing to its unique physical attributes such as large surface areas, tunable lengths and economic to use (Zeng et al., 2016). CNT based biosensors are the promising detectors recently explored for CP detection. A multi-walled carbon nanotube modified amperometric biosensor for CP detection was developed by Chauhan and Pundir (2011). In this sensor, maize acetylcholinesterase was immobilized onto iron oxide nanoparticles (Fe_3O_4NP) and carboxylated- multi-walled carbon nanotubes (Fe_3O_4/c-MWCNT) modified gold electrode (AuE). The biosensor fabrication and inhibition of immobilized acetylcholinesterase was achieved by an efficient chemical method (Fig. 3A). The SEM image of electrode showed the featureless morphology of bare Au electrode (Fig. 3B), distribution of Fe_3O_4NP/c-MWCNT (Fig. 3C) and immobilization of AChE onto the surface of modified Au electrode (Fig. 3D). Fig. 3E showed a range of the impedance spectroscopy for the bare and modified electrodes. Results indicated c-MWCNT coated Fe_3O_4 modified Au electrode showed good electron conducting properties and confirmed the successful assemblage on the electrode. Electrochemical characterization of the AChE–Fe_3O_4NP/c-MWCNT biosensor was also evaluated by cyclic voltammograms, which suggested that the presence of Fe_3O_4NP on the surface of AuE increased the electron transfer rate due to the large surface area and high conductivity (Fig. 3F). The changes in differential pulse voltammetry responses of the proposed biosensor were used to detect CP. This method offered a detection limit of 3.50×10^{-5} µg mL^{-1} for CP. In spite of the spectacular properties of CNTs, their direct utilization is restricted by poor dispersibility in polar solvents, which results in aggregation of CNTs (Bagheri et al., 2010).

5.1.4.2. Graphene based biosensors

Graphene is characterized by a single atom wide layer of carbon, thinnest known material, transparent, strongest ever estimated substance and incredibly flexible (Bollella et al., 2017). Graphene based biosensors targeting CP are particularly prominent because of their intrinsic properties of high electrical conductivity, surface area, and exceptional thermal and mechanical characteristics (Shan et al., 2010). These unique features facilitate highly sensitive detection and long-lasting stability in the fabricated biosensors (Lawal, 2018). In graphene based biosensors, graphene is used for the immobilization of biomolecules or for the detection of the target compound. Liu et al. (2011a) designed a novel biosensor based on immobilizing AchE on 3-carboxyphenylboronic acid (CPBA), reduced graphene oxide (RGO) and gold nanocomposites (AuNPs) modified electrode. Fig 4A represents the integration of CPBA, RGO and AuNPs in the electrode assembly. SEM image revealed successful immobilization of AuNPs on the surface of RGO (Fig. 4B). The Electrochemical impedance spectroscopy (EIS) results suggested that the RGO film increased the charge transfer rate of the modified electrode (Fig. 4C). Cyclic voltammograms of electrodes suggested that the synergy of RGO and AuNPs further improved the conductivity (Fig. 4D-E). Changes in amperometric responses of the fabricated biosensors due to alteration in oxidation current were used to detect CP. The developed biosensor observed to own good sensitivity for CP. Although sensitive, graphene based biosensors may suffer from disadvantages like covering of active sites available for electrocatalysis due to irreversible aggregation of graphene sheets, which can affect the efficiency of the sensing device (Bo et al., 2017).

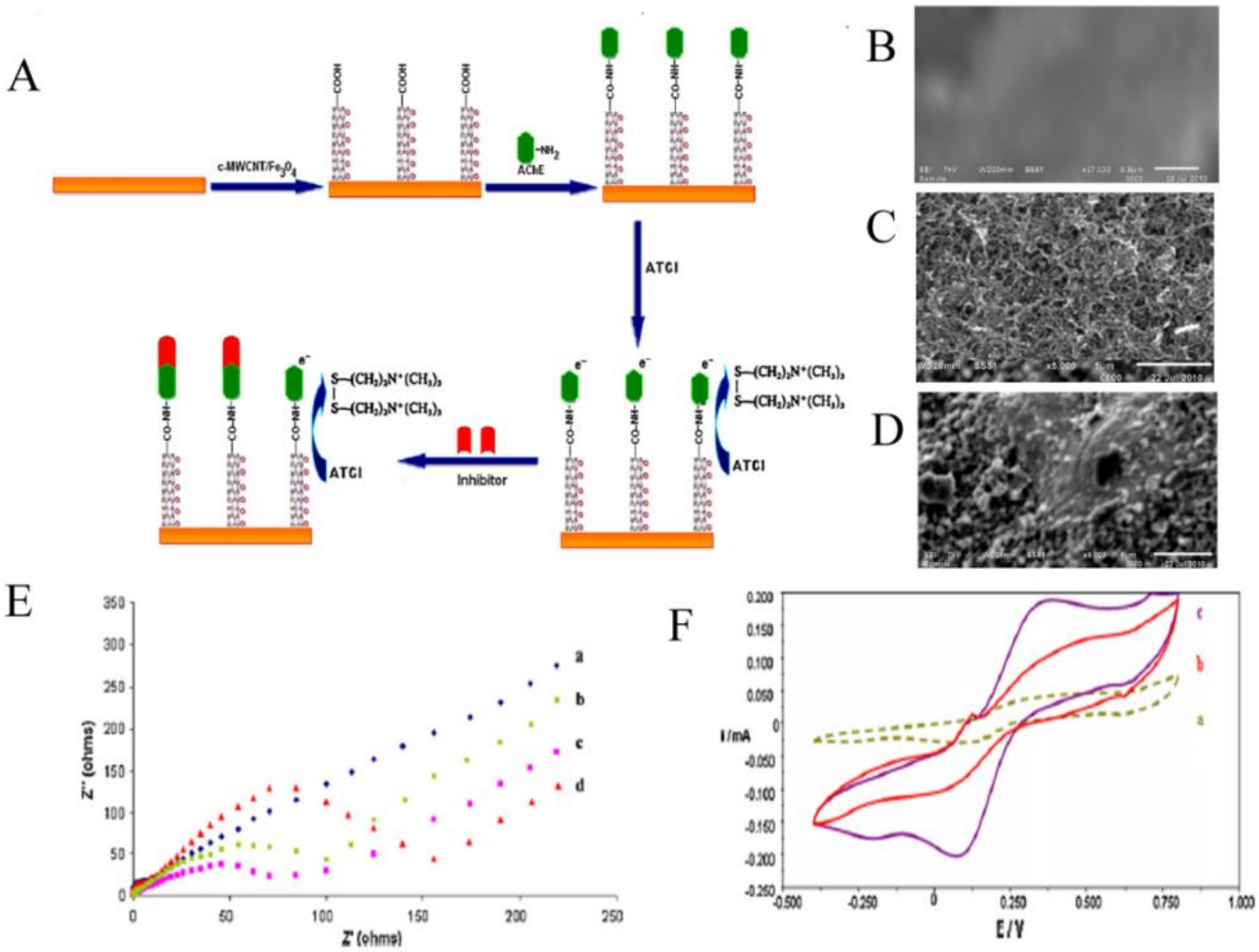

Fig. 3. (A) Schematic representation of the stepwise process of biosensor construction and its functions. Typical SEM image of (B) bare Au electrode (C) Fe_3O_4NP/c-MWCNT/Au electrode (D) AChE/Fe_3O_4NP/c-MWCNT/Au electrode. (E) impedance spectroscopy plot of (a) bare AuE, (b) c-MWCNT/AuE, (c) Fe_3O_4NP/c-MWCNT/AuE (d) AChE–Fe_3O_4NP/c-MWCNT/AuE. (F) Cyclic voltammograms of (a) AuE (b) Fe_3O_4NP/c-MWCNT/AuE (c) AChE–Fe_3O_4NP/c-MWCNT/AuE. Reproduced with permission from Ref. Chauhan and Pundir, 2011, Copyright 2011 Elsevier.

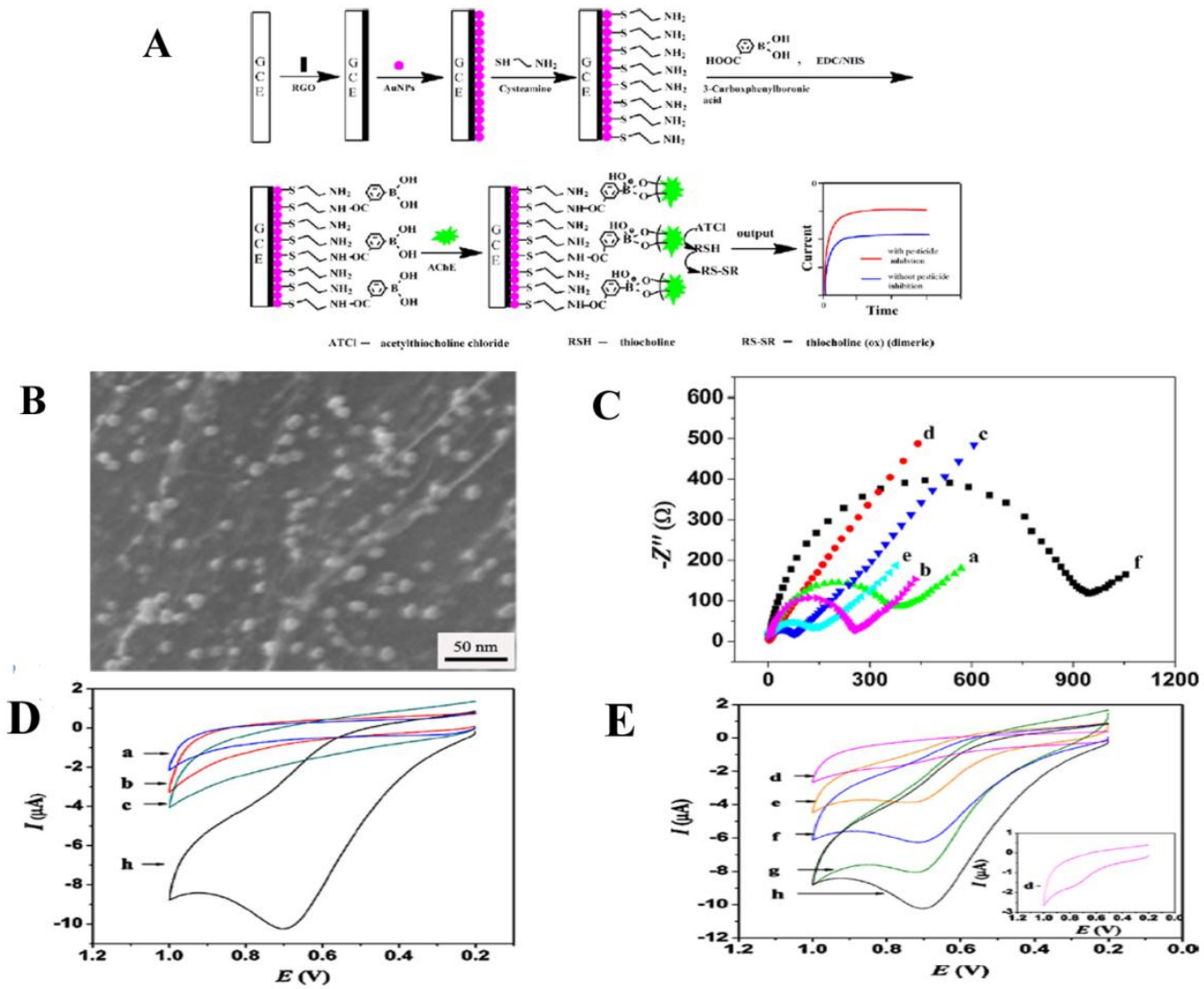

Fig. 4. (A) Schematic illustration of the preparation of AChE/CPBA/AuNPs/RGO-CS/GCE. (B) SEM image of AuNPs/RGO-CS/GCE. (C) EIS nyquist plot of (a) Bare GCE, (b) CS/GCE, (c) RGO-CS/GCE, (d) AuNPs/RGO-CS/GCE, (e) CPBA/AuNPs/RGO-CS/GCE, (f) AChE/CPBA/AuNPs/RGO-CS/GCE. (D) Cyclic voltammograms of (a) GCE (c) AChE/CPBA/AuNPs/RGO-CS/GCE (b) blank PBS; and CPBA/AuNPs/RGO-CS/GCE, (h) AChE/CPBA/AuNPs/RGOCS/GCE. (E) Cyclic voltammograms of (d) AChE/GCE (e) AChE/CS/GCE (f) AChE/RGO-CS/GCE (g) AChE/CPBA/AuNPs/GCE and (h) AChE/CPBA/AuNPs/RGO-CS/GCE. Inset: magnification of curve d. Scan rate: 0.1 V s^{-1}. Reproduced with permission from Ref. Liu et al., 2011a, Copyright 2011 Elsevier.

5.1.4.3.Gel based biosensors

Gel based biosensors utilize sol–gel as an appropriate matrix for immobilization of biomolecules as it stabilizes their biological activity and ensures prevention of leakage outside of the catalytic layer (Andreescu et al., 2002). In this technique, the sol (or solution) transforms into a gel-like mesh around the enzyme and includes solid and liquid phase (Blackman and Parkin,1997). Ionic liquids (ILs) have been characterized as molten salts, due to their liquid state below 100 ˚C and are composed of asymmetric cations and anions. ILs and their composites have been extensively used in various fields. Ionic liquid

(IL) was introduced in biosensor fabrication due to its novel characteristics such as high ionic conductivity, good electrochemical stability and wide electrochemical windows (Ho et al., 2014). A biosensor based on AChE and ionic liquid (IL)–carbon nanotubes gels were used for CP detection (Zamfir et al., 2011). Ionic liquid chosen for this study was 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF4]). Results indicated successful immobilization of AChE in sol–gel matrix and no interference from the IL was observed during CP detection. CP was detected by evaluating changes in the amperometric patterns of the proposed biosensor. The biosensor exhibited high stability and a low detection limit of 1.30×10^{-3} µg mL^{-1}. Nonetheless, there are still many constraints associated with the implementation of gel based biosensor such as low conductivity due to high viscosity, low electrochemical response rate due to the hygroscopicity of ILs, caused by water, involvement of diffusional limitation steps, and the complicated interaction between the catalyst–matrix (Wang and Hao, 2016).

5.1.4.4. Self assembled monolayers (SAMs) based biosensor

Self-assembled monolayers were usually prepared by the accumulation of balanced and closely packed monolayers onto the surface of a transducer (Wink et al., 1997). These monolayers contain amphiphile molecule with reversible affinity for the target molecule, at one end and a functional group at the terminal end (Pundir and Chauhan, 2012). In this process enzyme, immobilization is achieved using organic molecules as free anchorage group, which includes amines, disulfides, alkanesilanes, sulfides (Rathee et al., 2016). Long-chain (C12 or higher) n-alkylthiols or silanes are preferably used over shorter chains in order to ensure great extent of control over the molecular framework of the recognition interface (Gooding and Hibbert, 1999; Fragoso et al., 2008). An electrochemical biosensor for CP detection was developed by immobilizing the AChE enzyme on self-assembled monolayers of single walled carbon nanotubes (SWCNT) on gold (Viswanathan et al., 2009). Fig. 5A represents schematic of the fabrication, which comprised single strand oligonucleotide - single walled carbon nanotubes (ssDNA–SWCNT), Polyaniline (PANI) and AChE. Atomic force microscopy image showed a good adhesion of thiol terminated ssDNA–SWCNT on gold (Fig. 5B). The EIS results suggested that ssDNA–SWCNT SAM caused greater improvement in conductivity of the modified electrode as compared to plain ssDNA SAMs (Fig. 5C). Changes in the cyclic voltammetric patterns were used to detect CP. The detection limit of the biosensor was observed to be 3.5×10^{-7} µg mL^{-1}. The biosensor was perceived to be successful for CP detection, but such biosensor still faces some drawbacks such as possible electrode fouling and low reproducibility (Gooding and Hibbert, 1999; Rathee et al., 2016).

5.1.4.5. Polymer based biosensors

Conducting polymers are often qualified as synthetic metals due to their excellent electrical properties as encountered only in inorganic materials like silicon (Duke and Schein, 1980; Istamboulie et al., 2010). In polymer based biosensors, conducting polymers, such as polyacetylene, polypyrrole, polyindole, polythiophene, and polyaniline are used as enzyme immobilization matrix (Periasamy et al., 2009) or to enhance the conductivity of transducer (Chauhan et al., 2011). A novel, highly sensitive acetylcholinesterase based biosensor for CP detection has been constructed (Chauhan et al., 2011). In this biosensor immobilization matrices comprised of ZnS-nanoparticles (ZnSNPs) and poly (indole-5-carboxylic acid), fabricated on Au electrode (Fig. 6A). SEM study revealed the chemical immobilization of enzyme in the nanocomposite structure (Fig. 6B-D). EIS study of the modified electrode suggested obstruction in electron transfer at low frequencies because of the poor electrical conductivity of enzyme at low frequency (Fig. 6E). CP was detected with detection limit and accuracy percentage of 5.3×10^{-4} µg mL^{-1} and 95-100, respectively. In another study, poly (3,4-ethylenedioxythiophene) polycation (PEDOT) and a poly(styrenesulfonate) polyanion (PSS)-based conductive polymer was used for designing AChE-based biosensors (Istamboulie et al., 2010). The amperometric measurement showed a higher conductivity for the electrode modified with proposed mediator PEDOT:PSS than prevalent mediators like cobalt phthalocyanine or tetracyanoquinodimethane. Furthermore, amperometric method used for

CP-oxon detection indicated a reduction in current intensity after incubation with it. Biosensing device exhibited CP-oxon detection at concentrations as low as 1.4×10^{-3} µg mL^{-1}. Recently, a novel Polymeric membrane electrode biosensor coupled with molecularly imprinted solid-phase extraction was used for trace level analysis of CP in seawater. Potentiometric responses were further evaluated to detect CP with a detection limit of 1.08×10^{-5} µg mL^{-1} (Li et al., 2015). Despite the improvement in the domain of CP detection brought by rapid advancement in polymer based electrochemical sensors, the translation of the technology in real field analytical devices is still slow due to the challenges like high cost, complexities associated with fabrication, doping induced mechanical instability and shorter lifespan (Rathee et al., 2016).

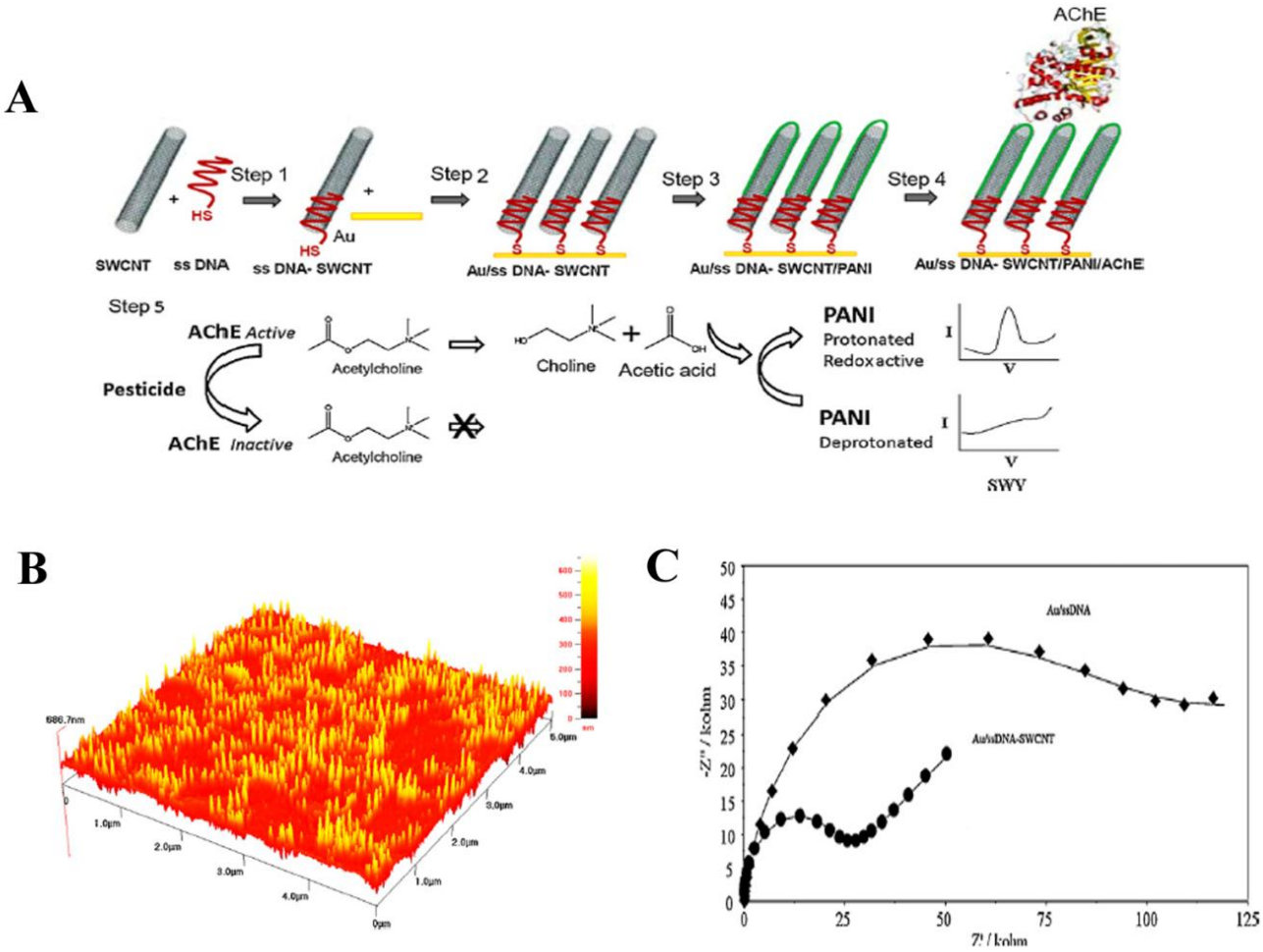

Fig. 5. (A) Schematic demonstration of the stepwise biosensor fabrication process (B) Typical Atomic force micrograph of ssDNA wrapped SWCNT SAMs on Au surface (C) EIS Nyquist plot of Au/ssDNA SAM and Au/ssDNA–SWCNT SAM. Reproduced with permission from Ref. Vishwanathan et al., 2009, Copyright 2009 Elsevier.

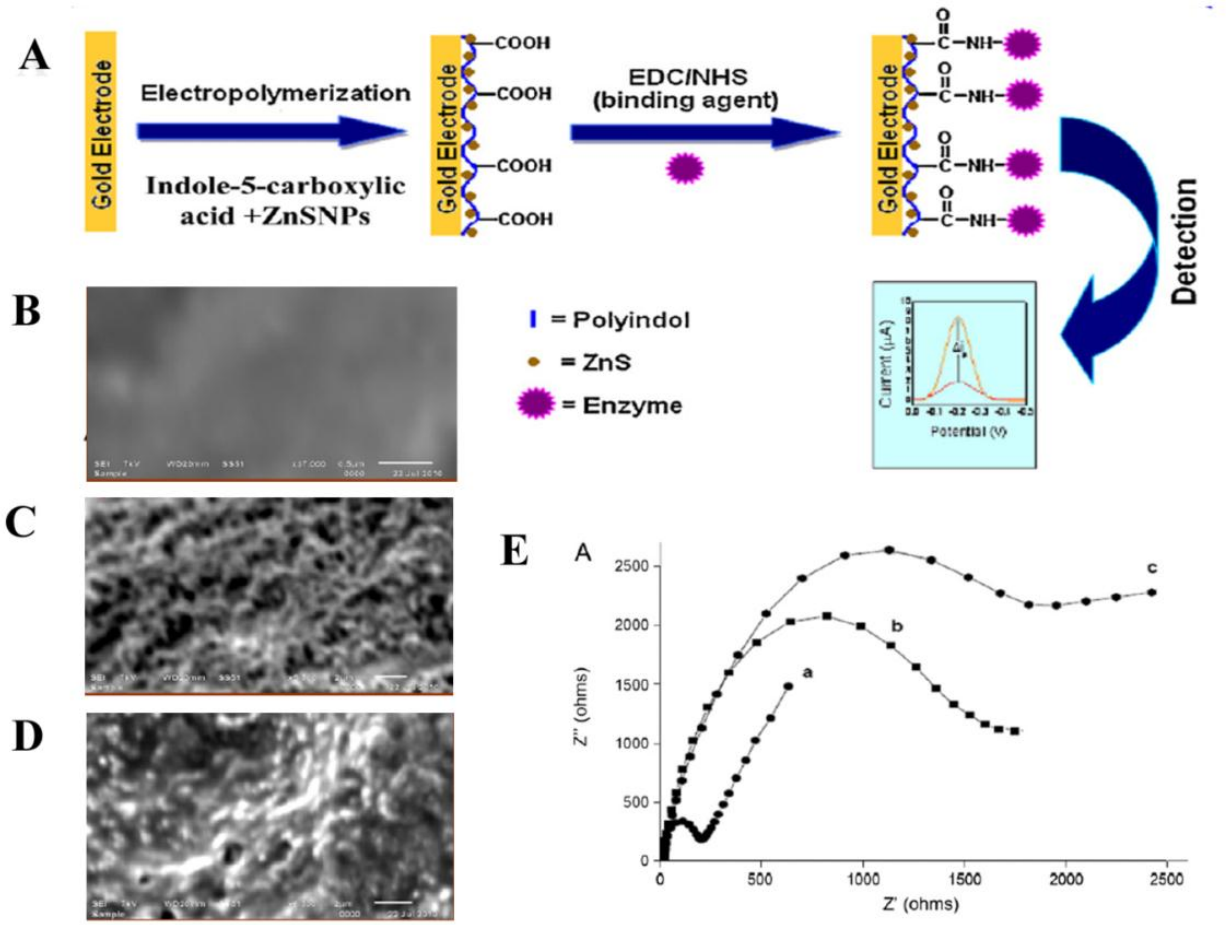

Fig. 6. (A) Schematic illustration of the biosensor fabrication and enzyme immobilization. SEM image of (B) bare Au electrode, (C) Pin5COOH/ZnS/AuE and (D) AChE-Pin5COOH/ZnS/AuE. (E) EIS plots of (a) bare AuE, (b) Pin5COOH/ZnS/AuE and (c) AChE-Pin5COOH/ZnS/AuE. Reproduced with permission from Ref. Chauhan et al., 2011, Copyright 2011 Elsevier.

5.1.4.6. Metal and metal oxide based biosensor

Nanostructured metal oxide has been used in biosensors because of their properties such as high surface area, nontoxicity, good biocompatibility, catalytic activity and chemical stability (Wang et al., 2014). Metal oxides nanoparticles offer a biocompatible environment for electrode performance by easing the immobilization of enzymes and amplify the enzyme sensitivity via facilitating electron transfer (Maduraiveeran et al., 2018). In a study, a novel biosensor based on a boron-doped diamond electrode qualified with gold nanoparticles-carbon spheres (AuNP-CS) nanocomposite was fabricated (Wei et al., 2014). Electrochemical behavior indicated an increase in peak current by 55% when AuNPs are incorporated in electrodes. Changes in differential pulse voltammetric patterns were used to detect CP with a detection limit of 4.55×10^{-8} µg mL^{-1}. In another study, Mayorga-Martinez et al. (2014) developed a biosensor based on iridium oxide nanoparticles (IrOx NPs) with screen printed electrodes. Transmission electron microscopy (TEM) of the synthesized IrOx NPs displayed their size range from 12.5 ± 2.5 nm (inset) and indicated spherical shape for most of the formed IrOx (Fig 7A). The EIS study indicated better conductivity owing to the high surface area provided by IrOx NPs (Fig. 7B). CP detection was demonstrated by monitoring changes in amperometric response of the fabricated biosensor. This

biosensor was observed to provide a low detection limit of 1.07×10^{-1} µg mL^{-1} for CP. Despite the high sensitivity and cost-effectiveness provided by current bio molecules based biosensors, their large scale use is relatively restrained by limitations such as the high cost for macromolecule isolation, low detection capability and the short useable lifespan of the identifying molecules (Daunert et al., 2000).

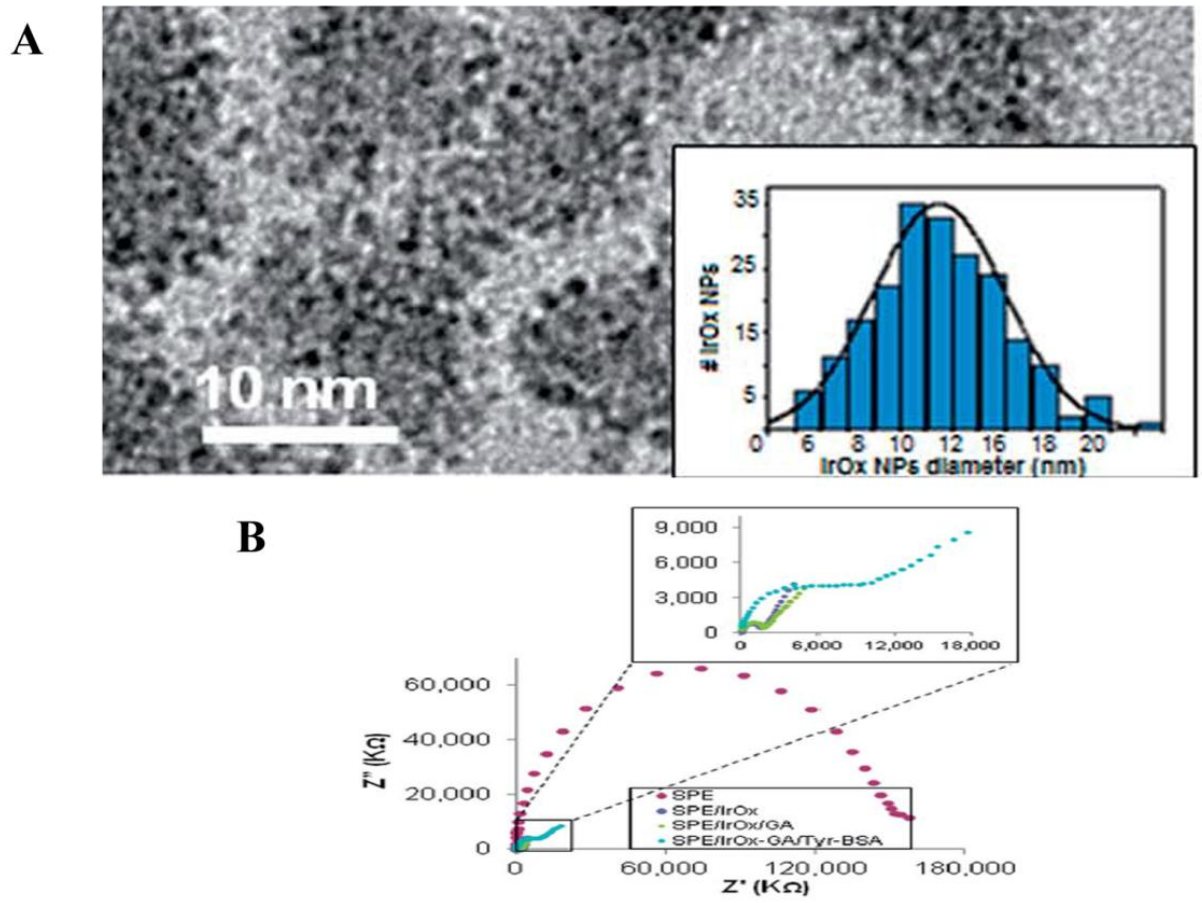

Fig. 7. (A) TEM images of iridium oxide nanoparticles (B) EIS studies of the modified electrodes surfaces. Reproduced with permission from Ref. Mayorga-Martinez et al., 2014, Copyright 2014 Royal society of chemistry.

5.2. Biomimetic molecule based biosensors

Recently, biomimetic molecule based biosensors have emerged as a suitable biological tool for the rapid screening and prompt detection of CP in the environment. Biomimetic recognition molecules have the obvious priority over other recognition elements because of low cost, marked selectivity, simplicity, and reliability (Xie et al., 2008). Technology for biomimetic molecule based biosensors has reached a new epoch by offering a broad spectrum of templates and functional monomers for bio sensing platform. Current approaches to the use of biomimetic molecule based biosensors involve nanoparticle based biosensor and molecular imprinted technology.

5.2.1. Nanomaterial-based biosensors

Nanomaterials have the size range of 1–100 nm and are considered most suitable candidates for biosensing applications because of their unique characteristics (Luo et al., 2006). For instance, they have

inherently high surface energy due to large surface-area-to-volume ratios, making them more active than their bulk material and providing more attachment sites for biological molecules than conventionally used polymeric membranes and fibrous materials (Wang et al., 2008). In addition, they possess high electrical conductivity, excellent magnetic attributes and catalytic activity (Kerman et al., 2008; Niemeyer, 2001). Moreover, they have the size comparable to biological molecules, which can increase the activity and lifespan of biological molecules after their immobilization on the surface of nanostructured materials (Fathil et al., 2015; Noor and Krull, 2014). Nanomaterial-based biosensors offer minimal susceptibility to matrix effects and better selectivity for the target compound than other biosensors (Zhang et al., 2014). An electrochemical biosensor based on nafion (NA) and Ag nanoparticles (AgNPs) was designed for CP detection (Guler et al., 2017). Fig. 8A represents the biosensor fabrication by integrating nafion-silver incorporated amine functionalized reduced graphene oxide (NA/Ag@rGO-NH_2/AChE). Transmission electron microscopy (TEM) revealed the presence of a clean and wrinkled surface on amine functionalized reduced graphene oxide sheets (Fig. 8B), providing the high surface area for attachment and distribution of AgNPs (Fig. 8C). The EIS curve represented by Nyquist plot indicated improvement in the conductivity of biosensor by AgNPs (Fig. 8D). Cyclic voltammograms of modified electrodes exhibited the magnificent electrochemical response to the thiocholine oxidation. The selectivity study on the biosensor response showed no measurable changes in the voltammetric peak current. Change in cyclic voltammetry characteristics of modified electrodes was used to evaluate the CP detection potential of the fabricated biosensor. This method offered the CP detection with a detection limit of 1.4×10^{-2} µg mL^{-1}. In another study, Mogha et al. (2016) constructed a novel biosensor based on reduced graphene oxide supported zirconium oxide nanoparticles. The electrochemical behavior of the AChE/ZrO_2/RGO electrodes showed two distinct linear ranges for CP detection, suggesting complete saturation of active sites of the enzyme. CP was detected by measuring changes in cyclic voltammetric characteristics of redox active ATCl. The sensor showed CP detection with a detection limit of 3.50×10^{-8} µg mL^{-1}. Although nanomaterial based biosensors offer a great prospect for CP detection, some defects potentially limit their efficiency and feasibility in practical applications. These involve lack of one step assay for CP detection and complex immobilization process in biosensor development (Kurbanoglu et al., 2017).

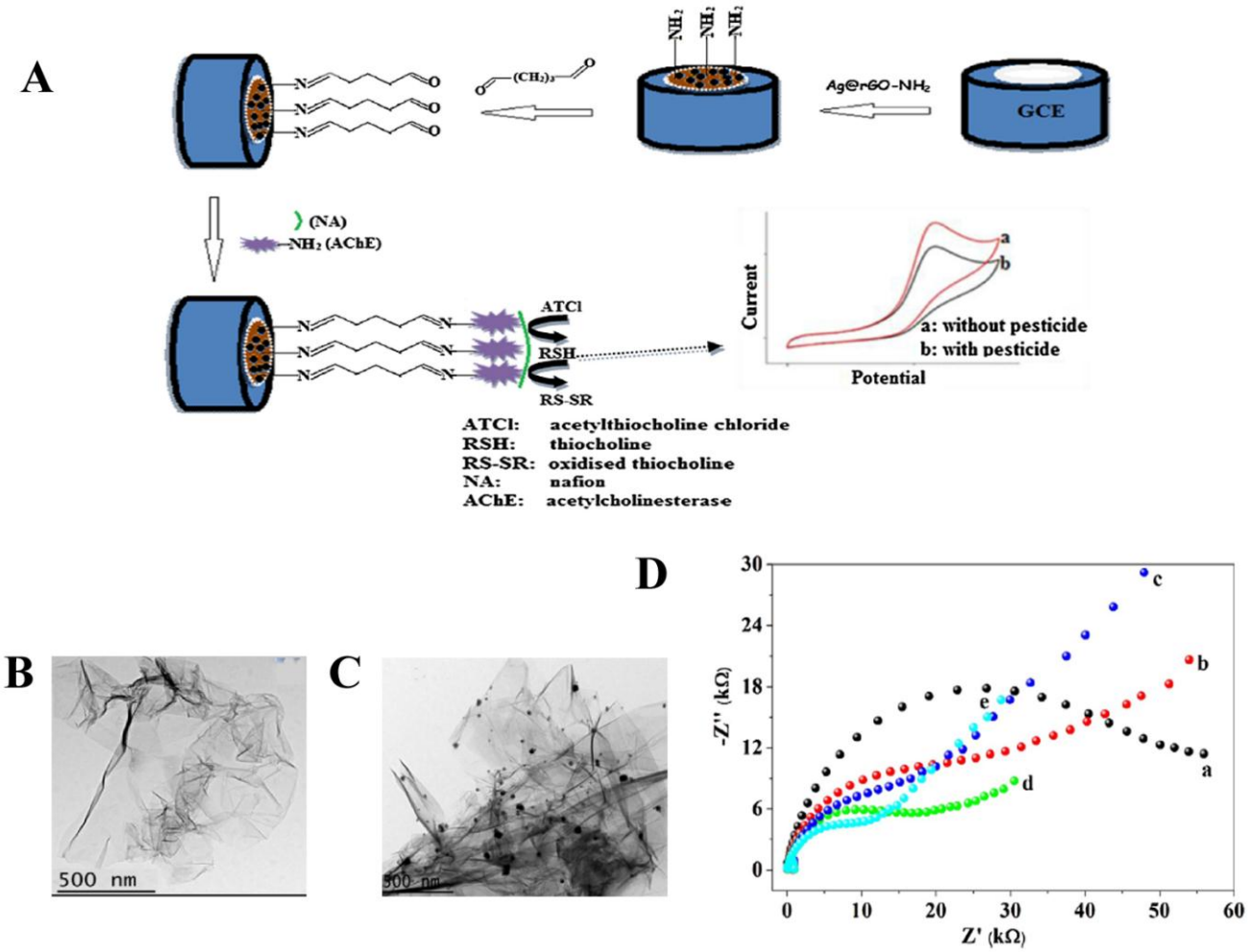

Fig. 8. (A) Schematic illustration of biosensor construction and its inhibition by the pesticide. TEM images of (B) rGO-NH2 (C) and Ag@rGO-NH2. (D) EIS plot of (a) NA/GCE, (b) NA/rGO-NH2/GCE, (c) NA/rGO-NH2/AChE/GCE, (d) NA/Ag@rGO-NH2/AChE/GCE, and (e) NA/Ag@rGO- NH2/GCE. Reproduced with permission from Ref. Guler et al., 2017, Copyright, 2017 Elsevier.

5.2.2. Molecular imprinted technology

Molecular imprinted technology offers a highly chemically stable, easily synthesizing and low cost detection approach for CP by generating specific recognition sites (Li et al., 2012; Silva et al., 2016). It involves cross linkage polymerization of the functional monomers and template mixture on the conducting support and formation of recognition sites corresponding to the morphology and functionality of the template (Say et al., 2005; Xie et al., 2011). A photo-electrochemical sensor (PEC) based on branched titanium dioxide nanorods (B-TiO_2 NRs) amended with a molecularly imprinted polymer (MIP) was fabricated for efficient detection of CP (Sun et al., 2017). B-TiO_2 NRs were used as photoactive materials that can be used to generate photocurrent owing to their high photoelectric activity under UV irradiation. The whole fabrication process comprised the integration of B-TiO_2 NRs grown vertically on FTO substrate, electropolymerization of MIP and removal of CP (Fig. 9A). Fig. 9B represents interactions of a hydrogen bond between ATP and CP. Fig. 9 C-E confirmed the formation of thin MIP films on the surface of B-TiO_2 NRs. Cyclic voltagramms of imprinted MIP-B-TiO_2 and non-imprinted

(NIP) electrode clearly indicated that the presence of imprinting features on the surface of modified electrode increased the conductivity (Fig. 9 F-G). Cyclic voltammetric measurements were carried out to evaluate the efficiency of proposed PEC sensing platform to detect CP sensitively (Fig. 9). The photocurrent response in the differently modified sensing platforms is studied (Fig. 9H). Almost no photocurrent is observed from bare FTO (curve f, fig. 9H). However, a considerable increase in photocurrent was observed after modification with B-TiO_2 NRs (curve a, fig. 9H). Incorporation of insulating layers between the excitation source and detection signal can hinder the harvesting of light and electron transfer and thus cause the reduction in background signal of PEC sensor (Li et al., 2016; Sun et al., 2017). Driven by this, NIP anchorage caused the photocurrent to decrease by forming an insulating layer (curve e, fig. 9H). Afterwards, removal of template resulted in the significant increase of photocurrent due to the exposure of B-TiO_2 NRs to ultraviolet light (curve b, fig. 9H). Furthermore, the photocurrent decreased with an increase in the concentration of CP from 0.1 ng mL^{-1} (curve c, fig. 9H) to 10 ng mL^{-1} (curve d, fig. 9H) indicating an obvious improvement in CP detection with PEC sensing platform. Measurement of amperometric intensity in the presence of interfering analogues indicated satisfactory selectivity of the proposed biosensor. This MIP-B-TiO_2 NRs electrode was shown to have a detection limit of 7.4×10^{-6} µg mL^{-1}. Although very interesting results using MIP-based electrochemical biosensor have indicated their application in the CP detection, with certain limitations are still in their early age. For instance, modification process of the electrode surface is very complex and time consuming. Moreover, the whole polymerization process for MIP development is rather perplexed and should be further improved (Gui et al., 2018).

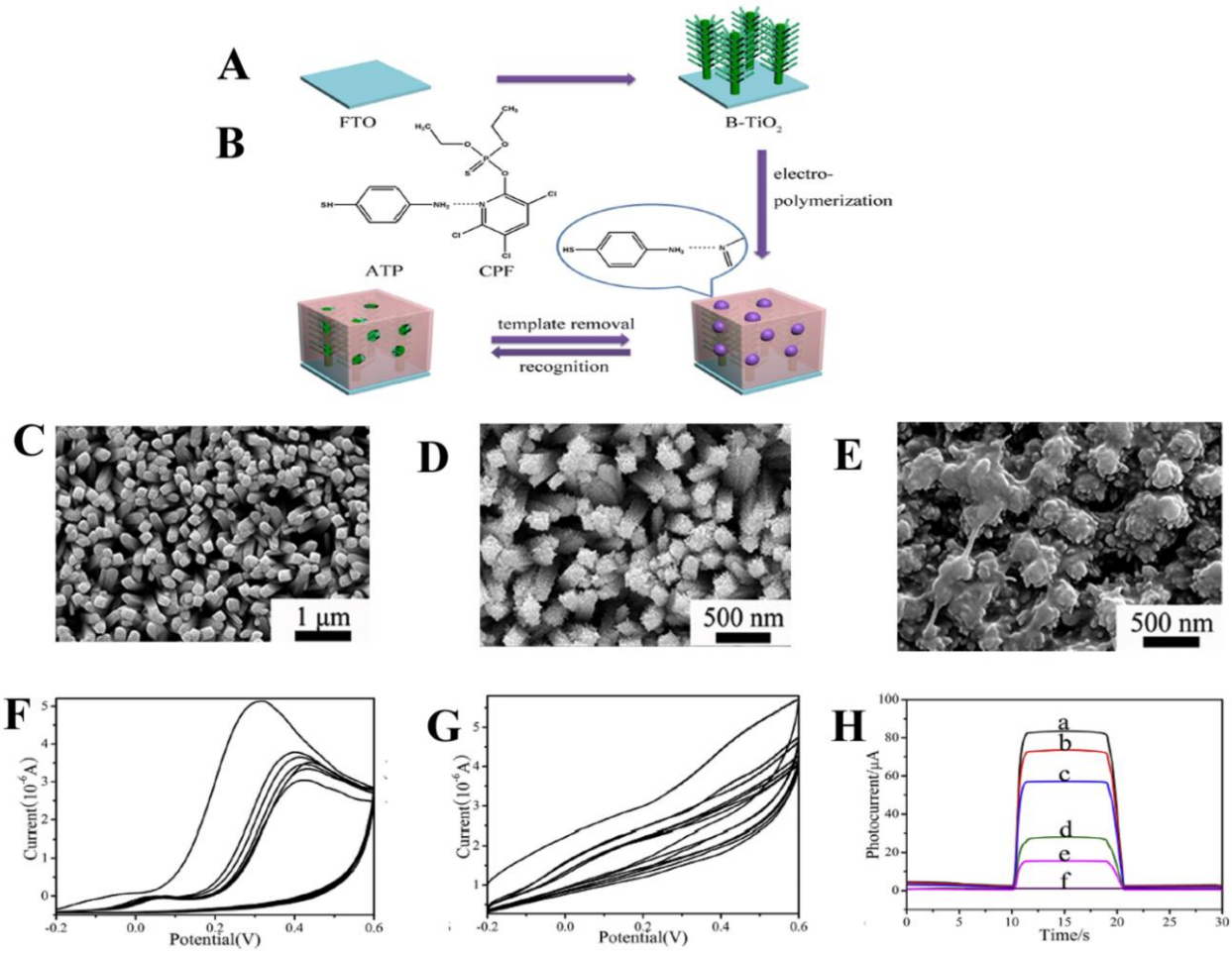

Fig.9. (A) Preparation of the PEC biosensor. (B) Schematic representation for the hydrogen bond interactions between ATP and CP. SEM images of (C) TiO_2 NRs (D) B-TiO_2 NRs (E) MIP-B-TiO_2 NRs. Cyclic voltammograms of (F) MIP (G) NIP. (H) Photocurrent response of (a) B-TiO_2 NRs, (b) MIP-B-TiO_2 NRs after template removal, (c) MIP-B-TiO_2 NRs after CP addition at concentration of 0.1 ng mL^{-1}, (d) MIP-B-TiO_2 NRs after CP addition at concentration of 10 ng mL^{-1},(e) NIP-B-TiO_2 NRs, (f) bare FTO in glucose. Reproduced with permission from Ref. Sun et al., 2017, Copyright 2017 Elsevier.

6. Summary and Conclusions

Electrochemical biosensors are proven to be a versatile technique for the detection of organophosphate pesticides like CP. Electrochemical biosensors can efficiently conquer the time consuming steps and requirement of trained personnel with attributes like sensitivity, portability, reliability and cost efficiency, which is hard to avoid in conventional instrumental techniques. In recent years, numerous developments in enzyme immobilization methods and fabrication techniques have opened new horizons for the evolution of various biomolecules based biosensors with desirable detection capability and miniaturization. However, in most of the cases, issues about stability, reproducibility, and complexities linked to fabrication process were some of the limitations needed attention. Nevertheless, the emergence of biomimetic molecules based biosensors has sparked a revolution in the field of CP detection with improved analytical behavior. Among these, nanomaterial-based biosensors have significantly proved to be of paramount interest in ensuring the sensitive, specific and throughput detection of CP.

7. Future Perspectives

Despite the considerable progress in the evolution of electrochemical biosensors for CP detection, many challenges and outlook still remain.

1. The application of this technology for CP detection in the field is still a challenge because of certain barriers, particularly in maintaining the stability of these biosensors. However, the stability of certain biosensors such as MIP based biosensors can be increased by integration of magnetic MIP complex and magnetic electrodes (Gui et al. 2018). In addition, in-field applicability of these techniques may be further increased by the applicability of advanced formats such as lab-on-a-chip and paper platforms to achieve detection with fewer assays steps (Kurbanoglu et al. 2017), that would allow on-site testing in a short time.
2. Molecular targets of CP for many non-target species are still unknown, which complicates its translation into simple bioanalytical devices. Therefore, the interaction of CP with cellular moieties is still unclear and needs further investigation.
3. Nanomaterial based biosensors used for CP detection exhibit complex immobilization process. Thus, the development of novel functionalized nanomaterials with improved selectivity and easier enzyme immobilization methods holds great promise in the upcoming era of biosensing devices.
4. Although potentiometric biosensors have a remarkable CP detection potential owing to their prominent selectivity and sensitivity, currently, most of these biosensors are still less studied. Use of potentiometric biosensors requires optimization of experimental conditions, which is quite laborious and time consuming. Furthermore, meticulous stabilization of reference electrode remains the major challenge that limits broad spectrum detection. Therefore, further studies are required to simplify the optimization schemes and develop rapid and efficient methods to achieve rigorous stabilization of reference electrodes that would expand the application of potentiometric biosensors in electrochemical sensing field.
5. The development of biosensing technologies for current CP detection methods has achieved considerable progress but further commercialization is hampered due to some technical barriers. Typical issues that remain unresolved in connection with commercial success are low reproducibility, poor analytical performance in real samples, complex detection procedures and high cost

(Scognamiglio et al. 2010; Justino et al. 2017). Therefore, further improvements in terms of minimizing matrix effect, ability to conduct continuous and remote measurements, validation parameters, competitive cost, and autonomy are necessary (Scognamiglio et al. 2010; Justino et al. 2017).

Booming research efforts hold hope for the development of new generation of electrochemical biosensors enabling safety testing in foods, beverage, and drinking water. Indeed, electrochemical biosensors will positively lead the researchers towards diverse opportunities with minimal investment.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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HIGHLIGHTS

- Organophosphate pesticide, chlorpyrifos contamination in several environmental matrices is of significant public health concern.
- An organophosphate pesticide, chlorpyrifos contamination around the world and its environmental impact is discussed.
- Various biomolecule and biomimetic molecule based electrochemical biosensors used to detect chlorpyrifos are identified and reviewed.
- Maintaining the stability of biosensors and use of nano-particles in sensor development are anticipated in the future.