“Development of Electrochemical Sensors for Chlorpyrifos Detection”

Thesis

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By

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Pragati

Pantnagar **(Pragati Joshi)**
June, 2018 **Authoress**

CERTIFICATE-I

This is to certify that the thesis entitled **“Development of Electrochemical Sensors for Chlorpyrifos Detection”**, submitted in partial fulfillment of the requirements for the degree of **Master of Science** with major in **Chemistry** of the College of Post Graduate Studies, G. B. Pant University of Agriculture & Technology, Pantnagar, is a record of *bona fide* research carried out by **Ms. Pragati Joshi, Id. No. 51092,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been acknowledged.

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Pantnagar
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CERTIFICATE-II

We, the undersigned, members of the Advisory Committee of **Ms. Pragati Joshi, Id. No. 51092,** a candidate for the degree of **MASTER OF SCIENCE** with major in **Chemistry** agree that the thesis entitled **“Development of Electrochemical Sensors for Chlorpyrifos Detection’’** may be submitted in partial fulfillment of the requirements for the degree.

Sameena

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Member

(Om Prakash)
Member

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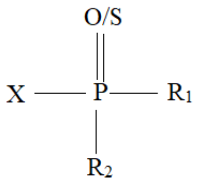
LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AE	Auxillary Electrode
AFM	Atomic Force Microscopy
ATCl	Acetyl thiocholine chloride
CHL	Chlorpyrifos
CNPE	Carbon Nano Paste Electrode
CNS	Central Nervous System
CNTs	Carbon Nano Tubes
CPE	Carbon Paste Electrode
CS	Chitosan
CV	Cyclic Voltammetry
DFP	Diisopropyl phosphor fluoridate
DPV	Differential Pulse Voltammetry
EC	Electrochemical
EIS	Electron Impedance Spectroscopy
FRA	Frequency Response Analyser
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GCE	Glassy Carbon Electrode
GNs	Graphene Nanosheets
HETP	Hexa Ethyl Tetra Phosphate
HPLC	High Performance Liquid Spectroscopy
LOD	Limit of Detection

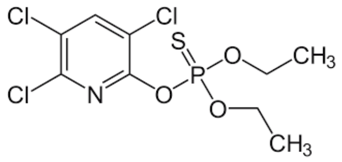
LSV	Linear Sweep Voltammetry
MS	Mass Spectrometry
MWCNTs	Multi Walled Carbon Nano Tubes
NC	Nano Composite
NP	Nano Particle
NF	Nafion
NMP	N- Methyl Pyrollidine
NPs	Nano Particles
OMPA	Octomethyl Pyrophosphotetramide
OP	Organophosphate
PATP	Poly Amino Thio Phenol
PBS	Phosphate Buffer Saline
PIN	Poly Indole
PNCs	Poly Nano Composites
QD	Quantum Dot
RE	Reference Electrode
RGO	Reduced Graphene Oxide
SEM	Scanning Electron Microscopy
SPS	Sulphonated Poly Sulphone
SSPE	Stainless Steel Plate Electrode
SWV	Square Wave Voltammetry
TEPP	Tetra Ethyl Pyro Phosphate
TLC	Thin Layer Chromatography
WE	Working Electrode
WC	Tungsten Carbide
XRD	X- Ray Diffraction

Introduction

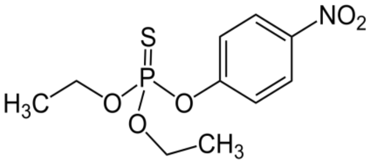
OPs are the esters, amides or thiol derivatives of phosphoric, phosphonic or phosphinic acids (Fig. 1a). They came into existence in 1932 and due to their acute toxicity OPs were used in 2nd World War as nerve agents **(Gupta, 2011; Worek *et al.*, 2005)**. OPs are widely used in agriculture for crop protection and pest control due to their high insecticidal activity, good efficiency and comparatively low persistence in the environment **(Singh, 2009)**. They have also been used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants and gasoline additives **(Guyton, 2001)**. OPs like DFP, TEPP and OMPA are used in the treatment of myasthenia gravis **(Lokesh *et al.*, 2017).** HETP was the first synthetically invented OP (in 1942) for use as an agricultural insecticide **(Kwong *et al.*, 2002; Tahara *et al.*, 2005)**. Some of the OPs work as potential insecticides and pesticides, which includes, chlorpyrifos, parathion, malathion, monocrotophos, diazinon, dichlorvos, dicrotophos, disulfoton, azinphos-methyl etc. (Fig. 1b).



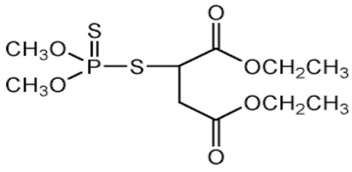
(a) General structure of OP



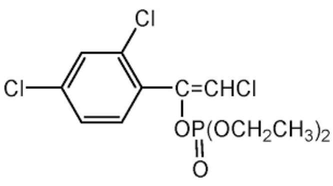
Chloropyrifos



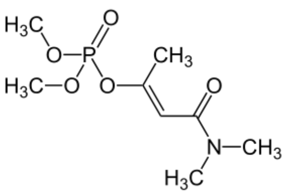
Parathion



Malathion



Dichlorvos



Dicrotophos

Azinphos- Methyl **Disulfoton**

Monocrotophos **Diazinon**

Fig. 1: Structures of some important OPs

OPs when sprayed on target crops get mixed into the soil and ground water only 0.1% reaches the specific target which is a matter of concern. Due to high solubility of OPs in water it contaminate lakes and rivers, and harm related organisms **(Aguiar *et al.,* 2004; Rand *et al.,* 1985).** OPs are highly toxic as they get efficiently absorbed by inhalation, ingestion and dermal penetration. OPs causes damage in CNS, as they do irreversible inhibition of AChE (catalyse hydrolysis of neurotransmitter acetylcholine to choline and acetic Acid) (Fig. 2). AChE is very important for CNS functioning and muscular responses, inhibition of AChE leads to disruption of nerve impulses transmission in vertebrates, birds, fishes and insects **(King *et al.,* 2015)**. Symptoms for OP poisoning in humans are headache, hypertension, hypotension, muscles twitching, nausea, diarrhea, vomiting, respiratory disorders, myocardial malfunctions and may lead to death in acute toxic condition **(Kamanvire *et al.,* 2004)**. To protect human health from this hazard there is a great need to develop selective, sensitive, fast, reliable methods for the detection of OPs.

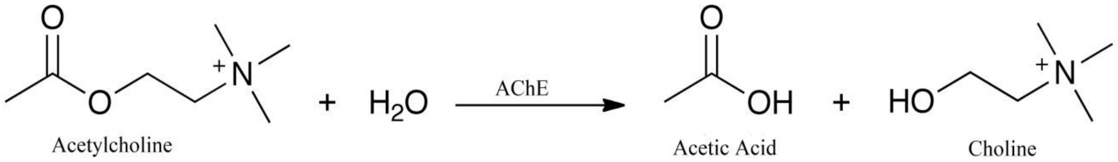


Fig 2: Schematic representation of mechanism of AChE inhibition due to OP

In the present investigation, studies were carried out on the quantification of CHL as model compound. CHL (o, o-diethyl-o-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) is a broad-spectrum, chlorinated OP that work as insecticide, acaricide and nematicide. It was first introduced and patented by Dow Chemical Company in 1965. It is synthesized by the reaction of 3,5,6- trichloropyridin-2-ol with o,o-diethyl phosphorochlorodithioate (**Simon *et al.*, 1998**). It is a white crystalline solid of mild mercaptan (thiol) odour, MW is 350.6 gmol^{-1}, have low water solubility (1.39 mgL^{-1}) and high soil sorption, 360 to 31. It is used at the rate of over 50,000 kg per year in Europe **(Manclus *et al.*, 1995)** and about 5 million kg per year in US **(Smolen *et al.*, 1997).** CHL persists in soil for 60-120 days and produces toxic effects to living organisms. It gets absorbed through skin, gut and pulmonary membranes by ingestion and contact. CHL affects immune system by disturbing AChE activity in developing foetus, mammalian cell cultures and lead to several neuro developmental disorders in humans and animals **(Eaton *et al.*, 2008).** There is an urgent need to detect the concentration of CHL at the low levels in environmental water and soil samples.

Many efforts have been made to develop sensitive, convenient, reliable, and effective methods for OPs analysis in environmental samples. Traditional complicated analytical methods for detection of OP compounds include GC, HPLC, capillary electrophoresis, MS and TLC **(Jia *et al.*, 2008; Chen *et al.*, 2006; Leandro *et al.*, 2006., Pérez-Ruiz *et al.*, 2005)**. Based on AChE, tyrosinase, CNTs a variety of enzyme based EC sensors were developed **(Zamfir *et al.*,2011; Chauhan *et al.*, 2011, Vishwanathan *et al.*, 2009; Liu *et al.*, 2011)**. Other methods involving amperometric biosensor, optical fibres, immunosensors, surface plasmon resonance and photochemical sensor were also reported for the detection of OP **(Kuswandi *et al.*, 2008; Mauriz *et al.*, 2006, Sun *et al.*, 2012; Wang *et al.*, 2013, Uygun *et al.*, 2013)**. But most of the above mentioned procedures are quite sophisticated and involve expensive instrumentation, have low sensitivity with the large consumption of chemicals and time. Alternatively in case of enzyme based sensors though high sensitivity is achieved but enzyme handling is quite complicated and require various steps like regeneration, incubation and immobilization. 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 the detection of OP and can be directly operated in environmental samples with minimum or no sample pretreatment steps **(Pedrosa *et al.*, 2003; Bebeselea *et al.*, 2008)**.

In the present EC study CV and SWV will be implemented for the detection of CHL. These methods involve three electrodes system working, reference and auxillary electrodes. CV has the capability of observing redox behaviour over a wide potential range and give explanation of kinetics for EC reactions at electrode surface **(Bard *et al.*, 2008; Bret *et al.*, 1993)**. It has extensive use in the fields of electrochemistry, biochemistry, inorganic chemistry and organic chemistry. The central parameters of a cyclic voltammogram (Fig. 4) are the magnitudes of anodic peak current (ipa), the cathodic peak current (ipc), the anodic peak potential (Epa) and cathodic peak potential (Epc). A potential is applied at the WE, which moves back and forth past the formal potential, current flows through the electrode that either oxidise or reduce the analyte. A cyclic voltammogram is obtained by applying a linear potential sweep to the WE. The magnitude of this current is proportional to the concentration of the analyte in solution. Equipments required in CV are three electrode potentiostat connected to three electrodes immersed in a test solution, potentiostat and a recording device **(Harnisch *et al.*, 2012)** The number of electrons transferred in the electrode reaction (n) for a reversible couple can be determined from the separation between the peak potentials. It specifies the relationship between potential of an electrode and concentration of the two species involved in a redox reaction (Eq.1).

$$E=E_0 + RT/NF \ln[Ox]/[Red] \qquad (1)$$

In CV, Randles-Sevcik equation (Eq. 2) describes the effect of scan rate on the peak current ip. For simple redox events, ip depends on scan rate. The diffusion coefficient of an electroactive species can be determined by this equation.

$$Ip = (2.69 \times 10^5)n^{3/2}AD^{1/2}Cv^{1/2} \qquad (2)$$

Fick's Law of Diffusion (Eq. 3) tells us that the current at any time is proportional to the concentration gradient of the reactant **(Streeter *et al.*,2008).**

$$I_t = NfaD_0(dC_0/dx)_{x=0,t} \qquad (3)$$

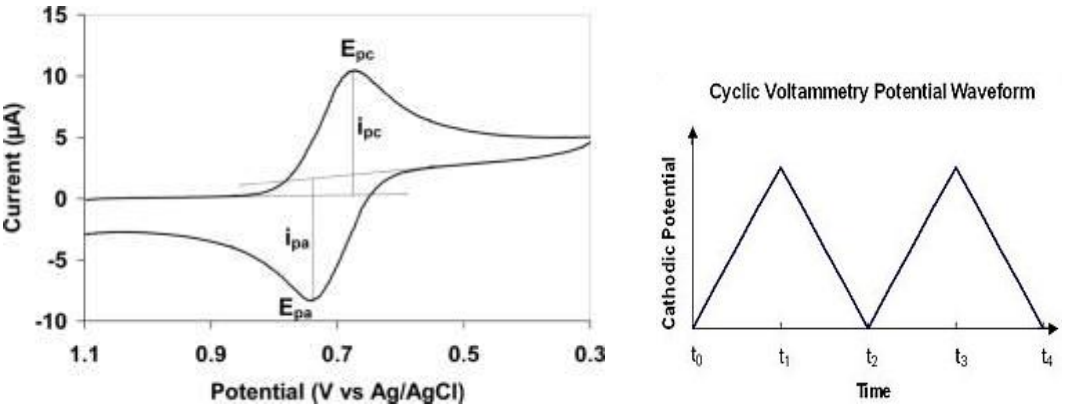


Fig.3 (a) Cyclic voltammogram (a graph between current and potential at three electrode system) (b) Cyclic voltammetry potential waveform

Over the past several years, there has been a reappearance of interest in electroanalytical techniques especially in pulse voltammetry. One of the most important electroanalytical technique among pulse voltammetric procedures is SWV, the major advantages of this technique include its very high selectivity and sensitivity **(Fisher *et al.,* 2016)**. It is more convenient in comparison to other techniques due to its extremely fast scan rates that resulted in drastic reduction in time of analysis. It is used in the pesticide analysis at very low detection value of the sensor. SWV is a differential technique in which potential waveform composed of a symmetrical square wave of constant amplitude superimposed on a base staircase potential **(Lovric, 2010).** It gives a plot of the difference in the current measured in forward (if) and reverse cycle (ir), plotted against the average potential of each waveform cycle. The differential current is recorded against the potential which results in a peak shaped voltammogram with accuracy in peak separation and rejection of background currents. As indicated from given Eq. 4 and Fig. 4, that the peak current increases with the increase in concentration of sample analyte and gives a calibration plot between peak current and concentration, that can be utilize for the quantification unknown analyte concentration.

$$I = [nFAD^{1/2}\, C^{b/\sqrt{\pi tp}}]\ \psi(dEs, Esw) \qquad \text{Eq. 4}$$

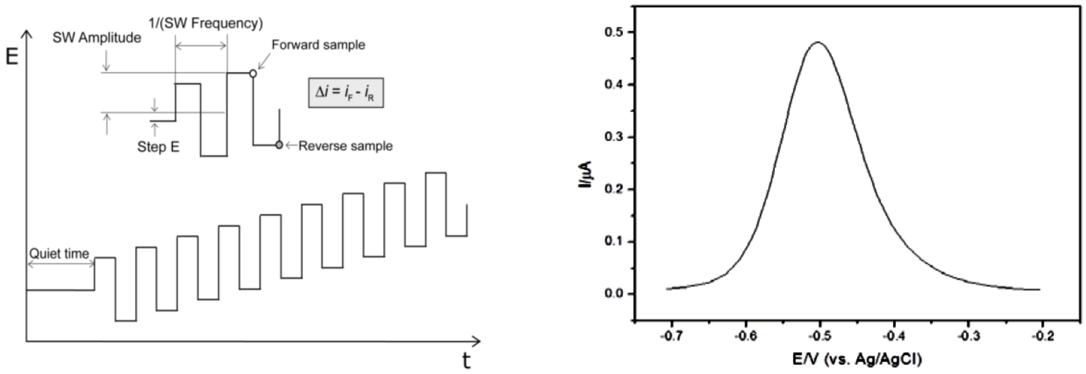


Fig 4(a): Potential waveform for SWV (b) Voltammogram of SWV (plot between current and potential)

Review of Literature

EC sensors offer several distinct advantages for the detection of OP including high sensitivity and selectivity, a wide linear range, portable size, rapid response time, simple operation, minimal space, power requirements, and low-cost instrumentation. In particular, such devices offer attractive avenues for interfacing, at the molecular level, bio molecular recognition events, and electronic signal-transduction processes. The efficiency of EC sensors for OP detection mainly depends on the modification of WE surface with different electroactive coating materials as describe in given literature review.

2. EC Sensors With Different Electrode Coating Materials

2.1 EC Sensors Based on Metal Nanocomposite Modified Electrodes

Metal oxides (Al_2O_3, SnO_2, TiO_2, CuO, RuO_2, MnO_2, V_2O_5 and MgO) remarkable properties like uniform size, identical size, wide surface area, excellent conductivity and well defined catalytic applications **(Plowman *et al.*, 2011, Devan *et al.*, 2012)**. They have been widely used as important electrode modification for the various EC applications including sensors, electronic devices and catalytic applications **(Bonnet *et al.*, 2003)**.

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} $molL^{-1}$ **(Li *et al.*, 2006)**.

For parathion detection an EC sensor was also developed by modified GCE with AuNPs and MWCNTs composite with CV electrodeposition method. For the characterization XRD 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} $molL^{-1}$ **(Zhang *et al.*, 2009)**.

Cobalt(II) oxide-decorated reduced graphene oxide electrode was reported for monitoring of carbaryl and carbofuran in food samples and LOD value for cabaryl and carbofuran was 7.5 μgL^{-1} and 4.2 μgL^{-1} respectively **(Wang *et al.*, 2014).**

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 has excellent conductive properties, so AChE had high affinity for ATCI which catalyze hydrolysis of ATCI. AChE biosensor formed have good selectivity, high stability and sensitivity and gives excellent response for CHL detection having detection limit 0.05 μgL^{-1} and linearity range 0.05 to 1.0×10^3 μgL^{-1} **(Chen *et al.*, 2015).**

Huang et al. modified GCE surfaces by using AuNPs and PdNPs combined with MWCNTs. The GCE surface was modified with 5 µL 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 $molL^{-1}$ H_2SO_4 solution containing 5.0 $mmolL^{-1}$ $HAuCl_4$. 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 6.0×10^{-5} $molL^{-1}$ with a LOD of 1.0×10^{-7} $molL^{-1}$. 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% **(Huang *et al.*, 2010).**

2.2 EC Sensors based on Polymers Modified Based Electrodes

For analysis of OPs, conducting polymers like polypyrrole, polyaniline, polyindole, poly acetylene, polythiophene, polyfuran, poly(para-phenylene), poly(*o*-phenylinediamine) and poly(3-hydroxylthiophene), are generally used as electrode coating materials in EC sensing methods **(Vakurov *et al.*, 2004; Eisert *et al.*, 1995)**.

Li 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 $nmoL^{-1}$ and satisfactory recoveries for water samples were achieved (between 90.3% and 94.7%) **(Li *et al.*, 2003)**.

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 isoproturon) were evaluated. The developed sensor exhibited good reproducibility and acceptable stability, thus providing a new promising tool for analysis of enzyme inhibitors **(Sundari *et al.*, 2011)**.

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 $molL^{-1}$ NaCl) was obtained when methiocarb in 0.1 $molL^{-1}$ H_2SO_4 was electrochemically characterized, which was used for its quantification. Linearity range was between 6.7×10^{-6} and 2.6×10^{-4} $molL^{-1}$ and a LOD of 2.0×10^{-6} $molL^{-1}$. 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 **(Inam *et al.*, 2013)**.

AuNPs coated GNs modified GCE electrode has been prepared for OP sensing. Methyl parathion was used as model compound. EIS was used for detecting the ability of electron transfer of the prepared electrodes. It was found that after coating of AuNPs on CS/GNs modified GCE, EC activity of electrode increases. A semicircle and a straight line were seen in case of bare electrode. After the fabrication of GCE with GNs there is rapid decrease in the thickness of semicircle, followed by successive decrease on further coatings. The voltammetric stripping analysis was carried out using CV and SWV. The L.O.D. and linearity range was found to be 0.6 $ngmL^{-1}$ and 0.001- 1.0 gmL^{-1} respectively **(Gong *et al.*, 2011)**.

Au/Pt NPs based sensor was developed in which amino glutardehyde was employed as crosslinker. Bimetallic NPs were found to be extremely useful due to their ability to enhance electron transfer rate (E_{tr}) and oxidation current. Characterisation was done by SEM, CV and EIS. LOD of aldicarb, paraoxon ethyl and sarin was found to be 40-60 µ $molL^{-1}$, 150-200 µn $molL^{-1}$ and 40-50 µn $molL^{-1}$ respectively. Interface features of the electrodes were characterised using EIS, frequency range and amplitude were set at 0.01 Hz-10 kHz and 0.2 V respectively. In the Nyquist plot semicircle shows (E_{tr}) at higher frequency and straight line indicates (E_{tr}) at lower frequency (**Upadhyay *et al.*, 2009)**.

An extremely sensitive RGO/NF composite sensor was developed and furthermore an exhaustion test of 100 bending cycles supports its authenticity. As prepared RGO/NF based electrodes are highly sensitive (10.7 $molL^{-1}$) with LOD 1.37 $\times 10^{-7}$ $molL^{-1}$. They exhibit prominent response time of <3 s. RGO/NF films displays low resistance, Nernstian behaviour and promote fast electron transfer **(Choi *et al.*, 2010).**

For the detection of methyl parathion a graphene/ZrO_2 based sensor was prepared. By lewis acid base interaction OP get bind to ZrO_2 NPs. The proposed sensor possess very low LOD of 0.1 $ngmL^{-1}$. The EC stripping response was exceptionally linear ranging from 0.5 $ngmL^{-1}$ to 0.1 $ngmL^{-1}$ **(Du *et al.*, 2007)**.

A reliable, sensitive and selective Al_2O_3 NPs comprised EC sensor was fabricated for the detection of OPs. It has been found to be a highly effectual sorbent due to its association with the phosphoric group of OP pesticide. Its LOD was found to be 0.035 $ngmL^{-1}$ with excellent linear calibration curves **(Wei *et al.*, 2012)**.

For the nanomolar detection of methyl parathion GCE was modified with graphene embedded with AuNPs. Synthesis of the sensor was done using green EC method. The fabricated electrode exhibits high electrocatalytic behavior for methyl parathion detection with LOD 0.82 $ngmL^{-1}$ and linearity range from 10 to 500 $ngmL^{-1}$ and 750 to 4000 $ngmL^{-1}$ **(Zhu *et al.*, 2014)**.

For the determination of malathion PAN/SWCNT/GO NCs were synthesized and employed with CV and DPV. The characterization was done through FT-IR, SEM, XRD and UV-Vis spectra. The respective detection limit and linearity range were 2.0×10^{-7} $molL^{-1}$ and 2.0×10^{-7} $molL^{-1}$ to 14.0×10^{-7} $molL^{-1}$ at pH 7. **(Ebrahim *et al.*, 2014)**.

2.3 EC Sensors Chlorpyrifos Detection

The sensitive detection of CHL was done on C_3N_4 NTs/GQDs/ GCE. Characterization of the prepared WE were carried out by CV and EIS. LOD and linearity range of the sensor were reported as 1.0×10^{-11} - 1.0×10^{-9} $molL^{-1}$ and $2.0 \times$ 10-^{12} $molL^{-1}$ respectively. EIS was performed on three different sets of electrodes which include bare GCE, GQDs modified GCE, and C_3N_4 NTs/GQDs/ GCE. Randles circuit was used as standard for the analysis of C_3N_4 NTs/GQDs/GCE, which includes constant CPE, solution resistance (Rs) and charge transfer resistance (Rct). The graph showed that Rct of bare electrode, GQDs/GCE and C_3N_4 NTs/GQDs/GCE was found to be 210 ohm, 160 ohm and 110 ohm respectively. Hence the catalytic activity and area of electrode increases on fabrication with C_3N_4 NTs/GQDs. (**Yola *et al.*, 2017).**

Y. Jiao *et al* designed a novel and ultrasensitive aptamer sensor for the quantitative detection of CHL. To improve the sensitivity of the aptasensor, mesoporous carbon functionalized by CS and ferrocene hybrid CS dispersed FeMWCNTs-CS were modified on the electrode surface. Under optimal conditions the designed aptasensor exhibited a wide linear range from 1 to 10^5 ngmL^{-1} with a low detection limit of 0.33 ngmL^{-1} (S/N = 3) for CHL **(Jiao *et al.*, 2016)**.

The cyclic voltammetric behaviour of CHL was investigated at a poly-3,4-ethylenedioxythiophene modified GCE. Effects of initial scan potential, square wave pulse amplitude, step potential and frequency were examined for the optimization of stripping conditions. The calibration plot was linear for 0.35–259.69 μgL^{-1} CHL **(Manisankar *et al.*, 2005).**

A novel Ag/Cu alloy nanoparticles and graphene NC paste electrode was fabricated and its EC activity was investigated using CV. A calibration plot of CHL was studied over concentration range, 0.01-100 nmolL^{-1} and detection limits of 4.0×10^{-12} molL^{-1} were achieved **(Sreedhar *et al.*, 2015)**.

PATP/AuNPs/GCE sensor using molecular self assembly method was prepared for CHL detection. The CV response of the proposed sensor is 3.2 times greater as compared to PATP/AuNPs. CV was recorded at a scan rate of 100 mVs^{-1} between -1.0 and +1.2 V. The EIS was scanned 0.01 M $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl solution at a potential of 0.25 V with a frequency ranging 1-105 Hz and a signal amplitude of 5 mV**(Xie *et al.*, 2010)**.

As it was found from the literature review that estimation of CHL is very important research area and relatively less work has been carried out for CHL estimation by EC methods (Table 1). So there is still scope and need for the detection of CHL by EC methods, and present investigation is in the direction of modification of WE with PIN/WC for the development of low cost and highly sensitive CHL EC sensor.

Table 1: Summary of reviewed studies on portable NP-based sensors for the detection of CHL

S.No.	**Target Analyte**	**Detection Technique**	**Electrode**	**Linearity Range**	**LOD**	**References**
1.	CHL	CV, SWV and EIS	C_3N_4NTs/GQDs/ GCE	1.0×10^{-11} -1.0×10^{-9} molL^{-1}	2.0×10^{-12} molL^{-1}	**Yola 2017**
2.	CHL	-	FeMWCNTs-CS	1 to 10^5 ngmL^{-1}	0.33 ngmL^{-1}	**Jiao *et al.*, 2016**
3.	CHL	CV	PEDOT/GCE	0.35 -259.69 μg L^{-1}	-	**Manisankar *et al.*, 2005**
4.	CHL	-	Ag/Cu/Graphene	0.01-100 nmolL^{-1}	4×10^{-12} molL^{-1}	**Sreedhar *et al.*, 2015**

Materials and Methods

3.1 Materials

CHL of purity (≥99.9%) was purchased from Sigma-Aldrich. The CHL dilutions were prepared in methanol. PBS was used as electrolyte. PBS was prepared using di-sodium hydrogen orthophosphate dihydrate (Na_2HPO_4. $2H_2O$) obtained from S.D. Fine Chem limited, sodium dihydrogen phosphate monohydrate (NaH_2PO_4. H_2O) was procured from Himedia and potassium chloride (KCl) from MP Biomedicals. PIN NC comprising various proportions of WC was directly used as provided by Department of Chemistry, G.B. Pant University of Agriculture and Technology, Pantnagar. A stock solution of CHL was prepared in 0.1M PBS. All reagents and chemicals were of analytical grade and were used without further purification. Throughout the experiment deionised water was used and all experiments were done at room temperature.

3.2 Preparation of PBS

Acidic (pH 4), basic (pH 9) and neutral (pH 7) buffer solutions were prepared by dissolving buffer capsules in 100 ml distilled water as per directions given on Himedia pH buffer capsules bottle. These standard buffer solutions were used to calibrate the pH meter and pH was adjusted using 0.1 M HCl and 0.1 M NaOH solutions. The EC response of CHL at PIN/WC SSPE was examined in 0.1 M PBS solution of pH 7.4. It was prepared by mixing 17.8 g $Na_2HPO_4.2H_2O$, 13.8 g $NaH_2PO_4.H_2O$ and 90 g KCl in 1 L distilled water.

3.3 Synthesis of SPS

SPS was synthesized using PSO resin (3.60 g) which was dissolved in DCM (35 ml) under continuous stirring at room temperature. A solution containing chlorosulfonic acid (6.50 % v/v) in DCM (15 ml) was added drop by drop for 2 h. The precipitated SPS was filtered and washed with NaOH (1.2 $molL^{-1}$) and deionized water until neutral water was obtained. Afterward, SPS was dried at 50°C overnight to remove traces of solvent and volatiles (Fig: 5) **(Mudila *et al.*, 2014).**

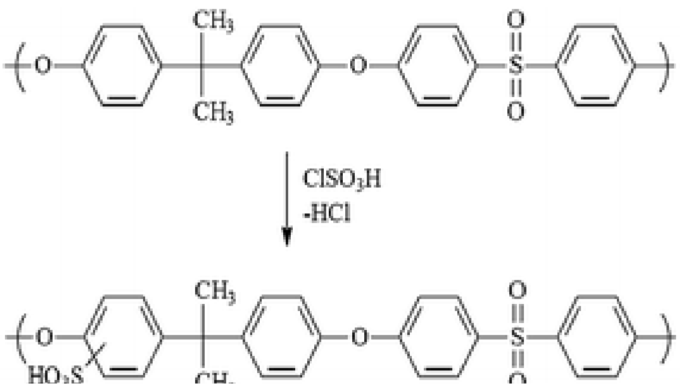


Figure 5: Scheme for SPS synthesis

3.4 Modification of WE Surface

Prior to modification SSPE of area 1 cm^2 was polished to a mirror like finish using emery paper and then rinsed with acetone. A solution of NMP (around 1 ml) and SPS (0.075 g) was stirred upto dissolution then graphite (0.0325 g) was added to it, followed by stirring for 2 hrs. Electroactive material (PIN/WC) was mixed with NMP, SPS and graphite solution with subsequent stirring for 2 hrs. Sonication was carried out over 30 minutes for thorough mixing of all components. Prepared solution of electroactive material then implemented for coating over SSPE and allowed to dry till fine coating was obtained (Fig: 6) **(Mudila *et al.*, 2014).**

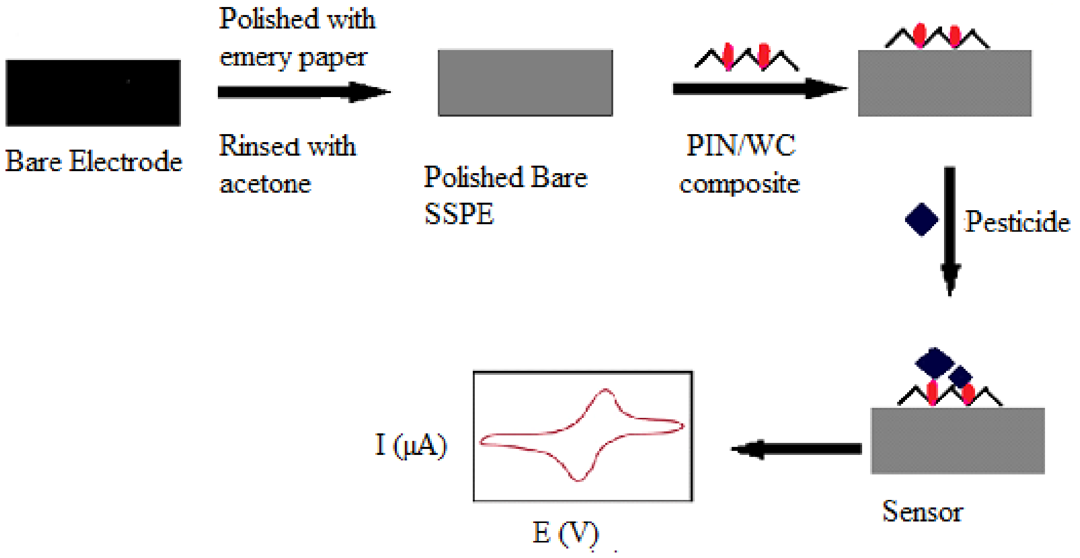


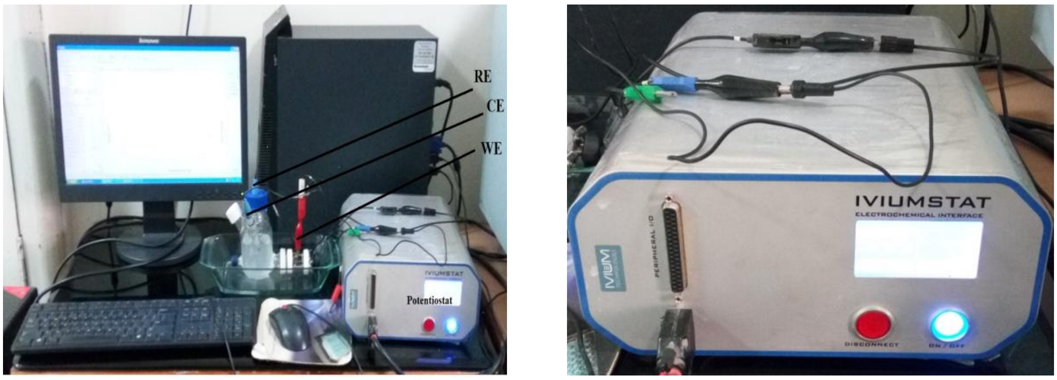
Figure 6: Stepwise modification of SSPE for the preparation of OP sensor

3.5 SEM

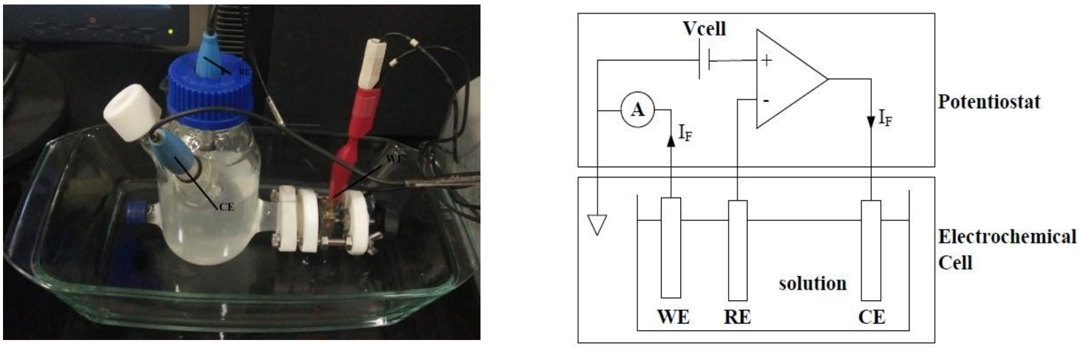
SEM was used for investigating surface framework of PIN/WC/SSPE with 5 and 30 (wt %). SEM was recorded on JEOL JSM 6610-LV instrument.

3.6 Electrochemical Procedures

CV, SWV and EIS were performed on IVIUM Potentiostat- Galvanostat (fig:7.a) using a triple electrode cell assembly (fig.6.c) dipped in PBS (0.1 M). For EC procedures, bare SSPE, 5% (w/w) and 30% (w/w) PIN/WC/SSPE were used as WE, Ag/AgCl was used as RE and Pt foil (1 cm^2) was employed as AE.



(a) Electrochemical Workstation (b) Potentiostat



(c) Triple Electrode Assembly

Fig 7: (a) Electrochemical workstation (b) Potentiostat (c) Triple electrode assembly

Results and Discussion

4.1 Solubility Studies of CHL

Solubility of CHL was checked in various solvent media under different conditions. It was found that CHL is insoluble in water and PBS at room temperature on mechanical stirring, heating and ultra sonication. CHL is partially soluble in ethanol and acetonitrile at room temperature on mechanical stirring, and found to be fairly soluble on heating with sonication. It was analysed that CHL is completely soluble in methanol on mechanical stirring at room temperature, heating as well as on ultra sonication. Therefore for subsequent EC studies, 0.1 M CHL stock solution was prepared in methanol (Table 2).

Table 2: Solubility studies of CHL in various solvent medium

S.No.	Solvent	Mechanical Stirring	Heat	Sonication
1.	Water	No	No	No
2.	PBS	No	No	No
3.	Ethanol	No	Yes	Yes
4.	Acetonitrile	No	Yes	Yes
5.	Methanol	Yes	Yes	Yes

4.2 Effect of pH on CHL Electrochemical Response

The EC response of CHL at PIN/WC SSPE was examined in 0.1 M PBS in the pH range of 4.0-10.0. It was found that best EC response was obtained in at pH 7.4, therefore all subsequent experiments of CHL estimation were performed in 0.1 M PBS at pH 7.4.

4.3 Surface Characteristics of Modified Electrodes

The surface characteristics of electrodes derived from PNCs at selected loading (wt%) of WC are shown through SEM under identical conditions (Fig. 8).

The SEM images of PIN/WC as shown in fig shows that WC was evenly fabricated on PIN matrix. The synergy between the constituents may be weak or strong which is regulated by the technique used for synthesis of the composite. The dark and bright phases corresponds to PIN and WC. PNCs prepared at 30 wt% of WC reveals rough surface with a polymer rich phase. The reason of surface roughness may be due to the non homogenized distribution of WC into polymer phase during the procedure of electrode preparation.

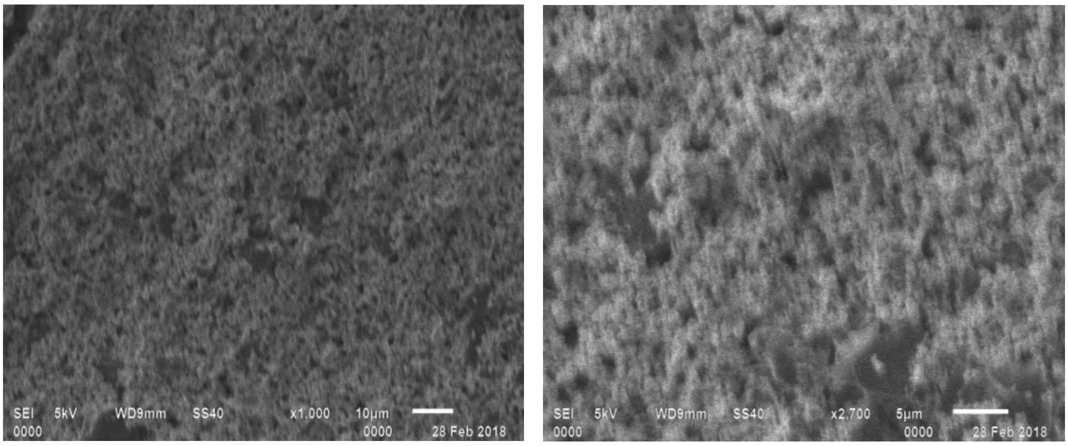


Fig 8: SEM images of 30 (wt%) PIN/WC/SSPE (a) At 1.0 KX (b) At 2.7 KX

4.4 Electrochemical Behaviour of Modified Electrode

4.4.a. Cyclic Voltammetry

The immobilization of functionalized layer on SSPE surface was confirmed through CV measurements. EC behaviour of CHL was studied in 0.1 M PBS at pH 7.4 by CV using PIN/WC-SSPE.

Figure 9 showed the cyclic voltammograms obtained for bare and 5 (wt%) WC in PIN/SSPE at a common scan rate of 40 mVs^{-1} with limit on the positive and negative end potentials at -0.11 and +0.55 V. There was a pair of well-defined redox peaks observed on the bare SSPE with the anodic (Epa) and cathodic (Epc) peak potential of -0.089 V and -0.418 V, while for modified SSPE -0.093 V and -0.43 V respectively. The peak current increased at modified PIN/WC SSPE surface, revealed that the coated material functioned as an electron-conducting tunnel and could promote the electron transfer between electrode surface and CHL thus generates synergy on EC properties. Reduction peak was observed as a result of electroreduction of pyridine ring of CHL as indicated in Fig 10.

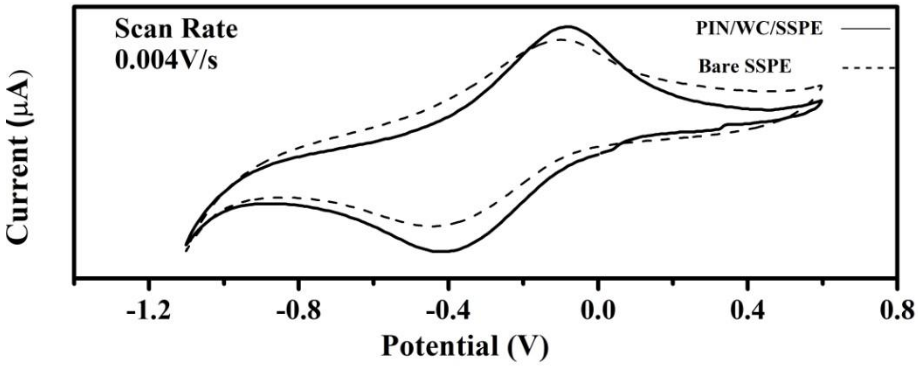


Fig 9: Comparison of CV curves of bare electrode and 5 (wt%) PIN/WC/SSPE

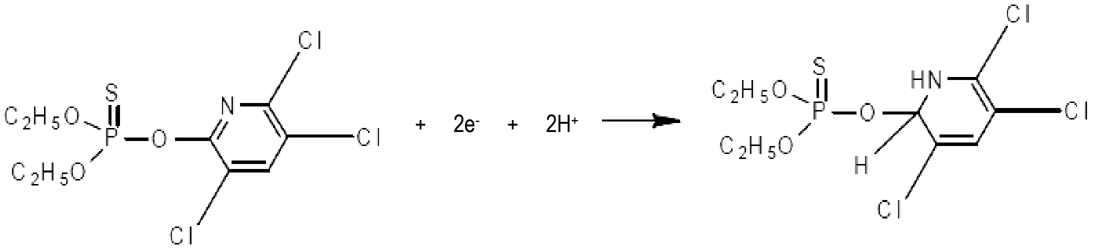


Fig.10: Electroreduction of pyridine ring of CHL.

4.4a.1 Effect of Scan rate on CV

For bare electrode (fig:11a) with frequent elevation in scan rate, the anodic peak current (Ipa) increases from 0.084 to 0.279 µA. The coinciding anodic peak potential was observed ranging from -0.089 to -0.013 V. At the same time there was reduction in cathodic peak current (Ipc) ranging -0.079 to -0.235 µA with corresponding cathodic peak potential (Epc) ranging -0.418 to -0.454 V.

For 5% PIN/WC/SSPE (fig:11b) with the increase in scan rate from 0.4 Vs^{-1} to 2 Vs^{-1}, there was subsequent increase in Ipa, Epa, Ipc and Epc ranging 0.111 to 0.329 µA, -0.093 to -0.065 V, -0.099 to -0.325 µA and -0.43 to -0.52 V respectively.

Further it was found that 30% PIN/WC (fig:11c) was independent of scan rate as no redox peak was observed in its cyclic voltammogram that reflects its capacitive behavior.

On comparing redox behavior of bare electrode and PIN/WC (containing 5% WC (w/w)) composite, it was found that the later shows better electrochemical behavior than bare electrode.

Table 3: Effect of scan rate on bare SSPE electrode

S.No.	Scan rate (V/s)	Ipa (µA)	Epa (V)	Ipc (µA)	Epc (V)
1	0.4	0.084	-0.089	-0.079	-0.418
2	0.6	0.120	-0.069	-0.112	-0.43
3	0.8	0.144	-0.057	-0.134	-0.438
4	1	0.185	-0.053	-0.179	-0.45
5	1.5	0.243	-0.025	-0.210	-0.462
6	2	0.279	-0.013	-0.235	-0.454

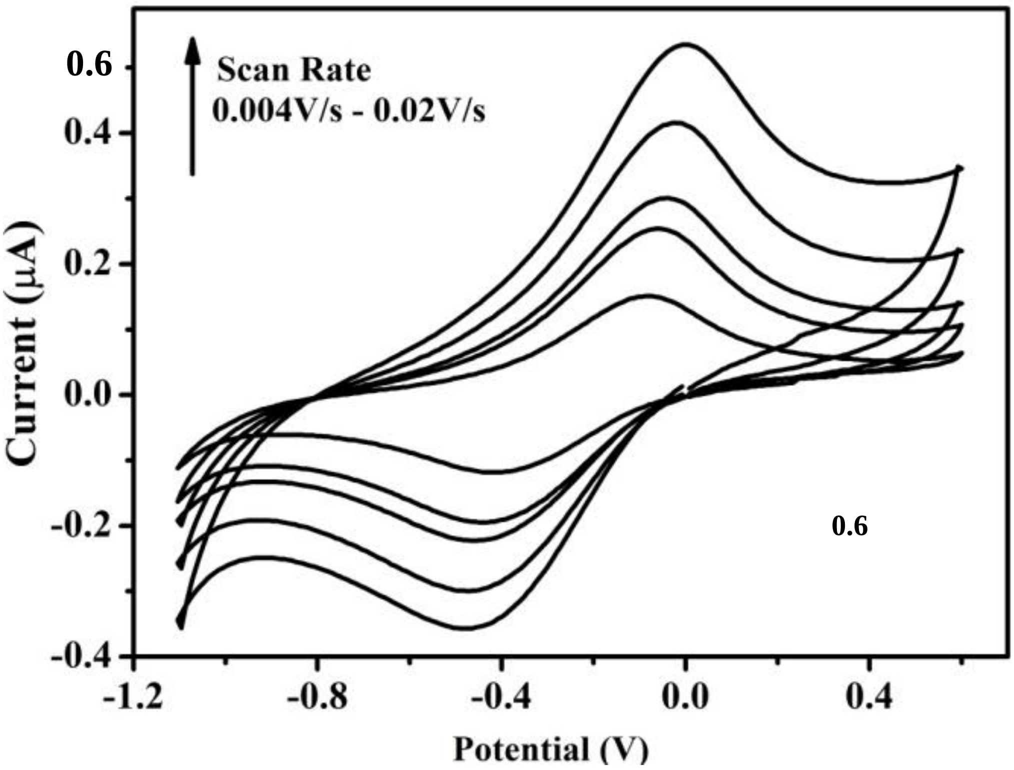


Fig 11 a: CV of bare SSPE vs. Ag/Ag$^+$ Electrode recorded in 0.1 M PBS (pH 7.4) at scan rates 0.4, 0.6, 0.8,1.0, 1.5 and 2.0V/s

Table 4: Effect of scan rate on 5 (wt%) electrode

S.No.	Scan rate (V/s)	Ipa (µA)	Epa (V)	Ipc (µA)	Epc (V)
1	0.4	0.111	-0.093	-0.099	-0.43
2	0.6	0.146	-0.093	-0.131	-0.462
3	0.8	0.188	-0.089	-0.19	-0.47
4	1	0.198	-0.085	-0.193	-0.478
5	1.5	0.288	-0.073	-0.255	-0.51
6	2	0.329	-0.065	-0.325	-0.522

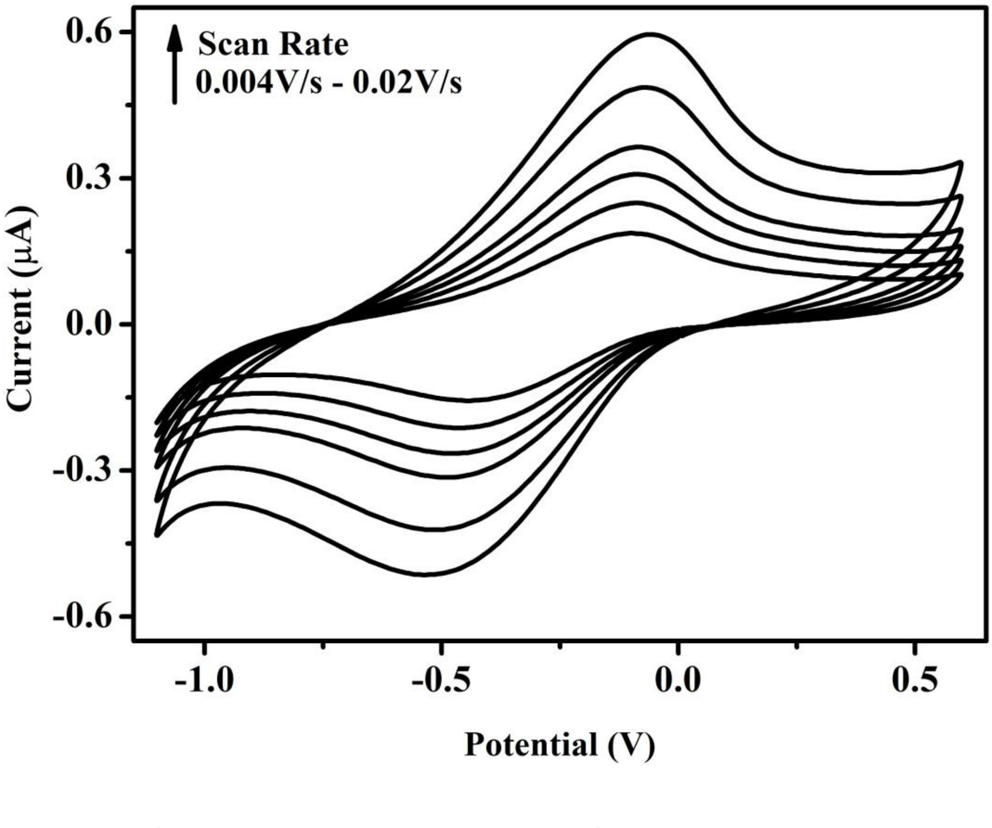


Fig 11 b: CV of 5% (w/w) PIN/WC composite fabricated SSPE recorded in 0.01 M PBS (pH 7.4) at scan rates 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0V/s

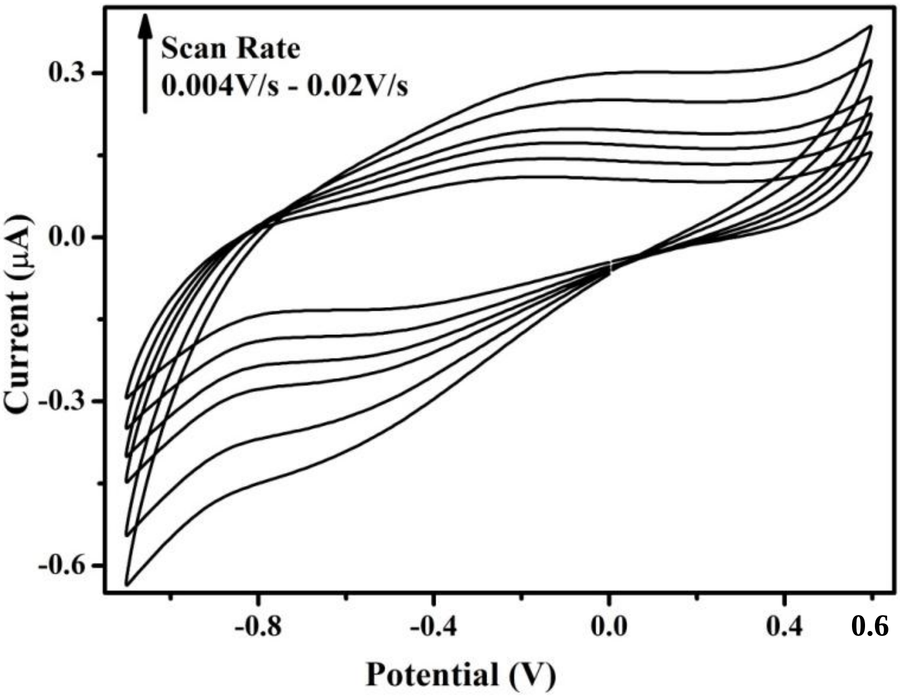


Fig 11c: CV of 30% (w/w) PIN/WC composite fabricated SSPE recorded in 0.1 M PBS

4.4.b Evaluation of Cs and Related PNCs.

The CV of samples were scanned at scan rate ranging 0.001 to 0.2 V/s, at 1 µA and various voltage compliances (Table:4).The C_S, is calculated from CV according to Eq. (5):

$$C_S=C/m \tag{5}$$

Where, C is capacitance of electrode, and *m* is the mass of the active material (0.04±0.01 mg) in various samples.

The capacitance of electrode (C) is calculated as according to Eq. (6):

$$C = Q/\Delta V \tag{6}$$

Here, ΔV is potential difference, Q is charge, and in turn calculated as:

$$Q= I\times dt \tag{7}$$

Where, *I* is current and is the average of anodic current (I_a) and cathodic current (I_c):

$$I=I_a+|I_c|/2 \tag{8}$$

*d*t is the time, and is calculated according to Eq. (5):

$$dt = \text{E-step/scan rate} \qquad (9)$$

The overall equation for calculating value of specific capacitance (C_S) of the active material can be written as, C_S=I/m (dV/dt) **(Ramya *et al.*, 2013)**.

Table: 5 Estimation of capacitance of 30% PIN/WC/SSPE

S.No.	Ic	Ia	(Ia+ Ic)/2	dt = Estep/ Scan Rate	Q= I× dt	C= Q/V	Cs= C/m
1.	-7.93	18.30	13.12	0.20	2.62	1.54	38.5
2.	-28.9	26.43	26.43	0.13	2.64	1.55	38.75
3.	-34.16	31.52	31.52	0.10	3.15	1.85	46.25
4.	-38.85	36.06	36.06	0.08	2.88	1.69	42.25
5.	-48.52	45.39	45.39	0.053	2.40	1.41	35.25
6.	-57.84	53.96	53.96	0.04	2.15	1.26	31.5

4.4.c. Square Wave Voltammetry

For the detection of CHL, PIN/WC/SSPE sensor was designed. The SWV experiments have been performed in the potential range from -2 to 2 V using the following optimal parameters: pulse amplitude 2 mV, scan increment 20 mV and frequency 25 Hz. (Fig:12 (a), (b), (c)) shows typical SWV voltammograms of bare SSPE, PIN/WC/SSPE 5(wt%) and PIN/WC/SSPE 30(wt%) respectively in 0.1 M PBS (pH 7.4) with varying concentrations of CHL. The occurrence of WC in the PIN matrix increases the sensing current of CHL, which is due to the electrocatalytic activity of WC NPs. Table 5 shows the effect of different concentrations of CHL on the peak current of CHL. Later it was found that the with the increase in CHL concentration there is enhancement in the peak current from 6.204 to 7.808 in bare electrode (Fig: 12(a)), 7.699 to 8.99 in 5% PIN/WC/SSPE (Fig: 12(b)) and 9.892 to 11.90 in 30% PIN/WC/SSPE (Fig:12 (c)).

Calibration curves between peak current and concentration were used to calculate LOD and LOQ. LOQ = 10s/m and LOD =3.3s/m where s is intercept and m is slope of the calibration curve. The proposed sensor demonstrates acceptable quantification of CHL with LOD values. The sensor calibration curve was obtained by plotting the SWV peak current against the respective concentration. The calibration plots for quantification of CHL at bare electrode(Fig:13 (a)), 5(wt%) PIN/WC/SSPE (Fig:13 (b)) and 30 (wt%) PIN/WC/SSPE (Fig: 13(c)) were obtained by studying the effect of increase in CHL concentration on peak current.

The LOD obtained for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE are 4.8×10^{-8} $molL^{-1}$, 5.94×10^{-8} $molL^{-1}$ and 4.49×10^{-8} $molL^{-1}$. LOQ for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE are 14.5×10^{-8} $molL^{-1}$, 18×10^{-8} $molL^{-1}$ and 13.6×10^{-8} $molL^{-1}$respectively. The correlations between the concentration and peak current were in linear relation. The correlation coefficient r for bare, 5(wt%) and 30 (wt%) was 0.979, 0.982, 0.971 respectively indicating good correlation.

Table 6: Effect of CHL concentration on peak current of bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE

S.No.	Vol. of 0.01M CHL added in 180 ml PBS (μL)	Final concentration of CHL in cell (M)	Peak current at bare electrode (μA)	Peak current at 5(wt%) PIN/WC/SSPE (μA)	Peak current at 30 (wt%) PIN/WC/SSPE (μA)
1	100	55.5×10^{-7}	6.204	7.699	9.892
2	150	82.5×10^{-7}	6.451	7.782	10.25
3	200	110.0×10^{-7}	6.700	8.067	10.69
4	250	137.5×10^{-7}	6.906	8.444	11.10
5	300	165.0×10^{-7}	7.105	8.564	11.35
6	350	192.5×10^{-7}	7.425	8.70	11.70
7	400	220.0×10^{-7}	7.808	8.99	11.90

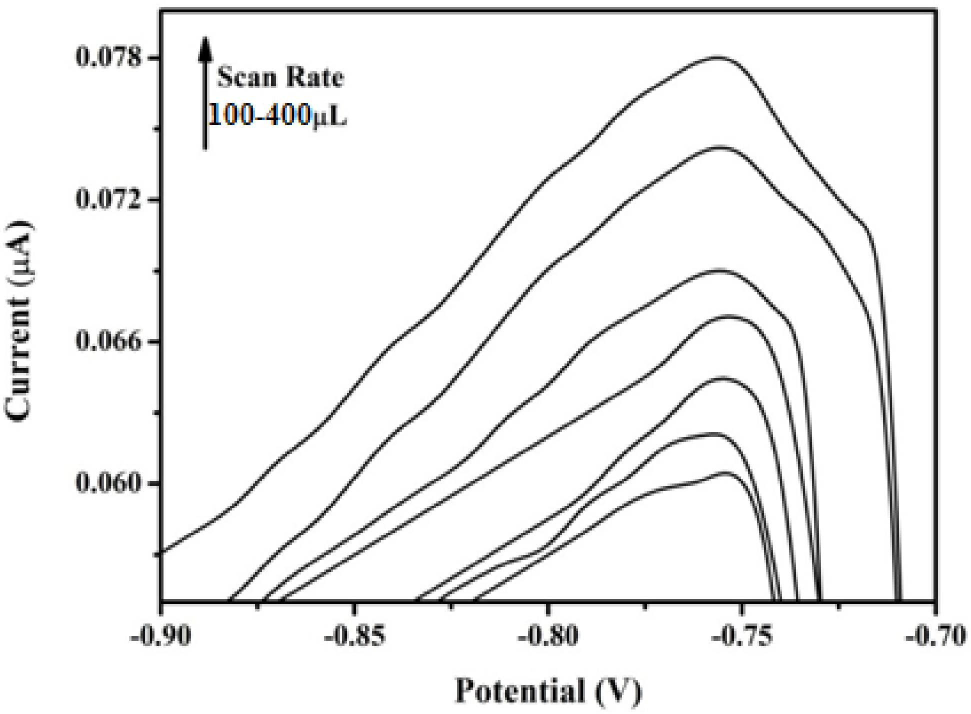


Fig 12 (a): SWV of CHL on bare SSPE

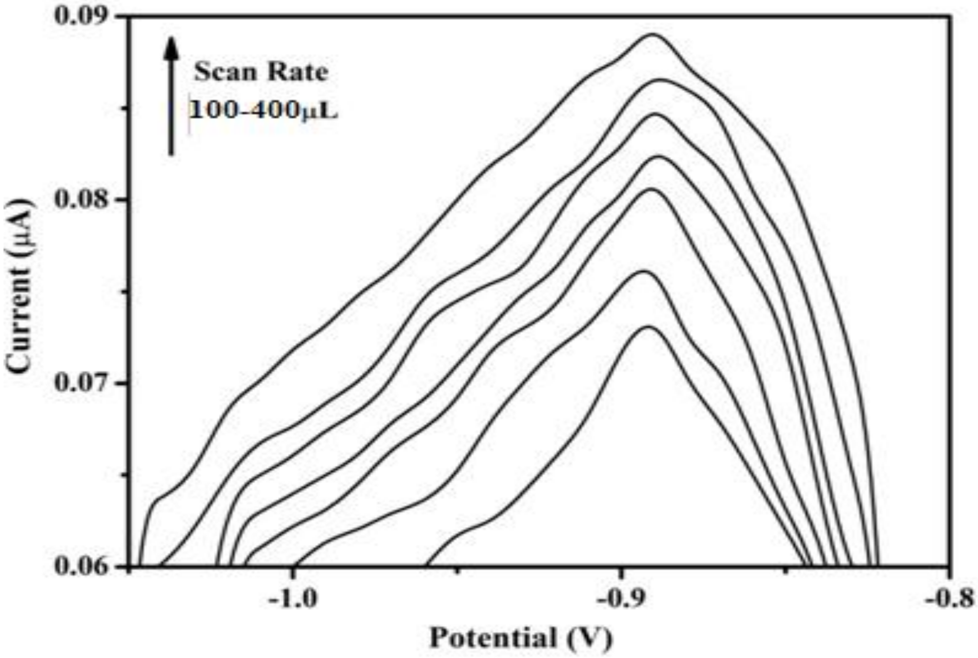


Fig 12 (b): SWV of CHL on 5% (w/w) PIN /WC/ SSPE

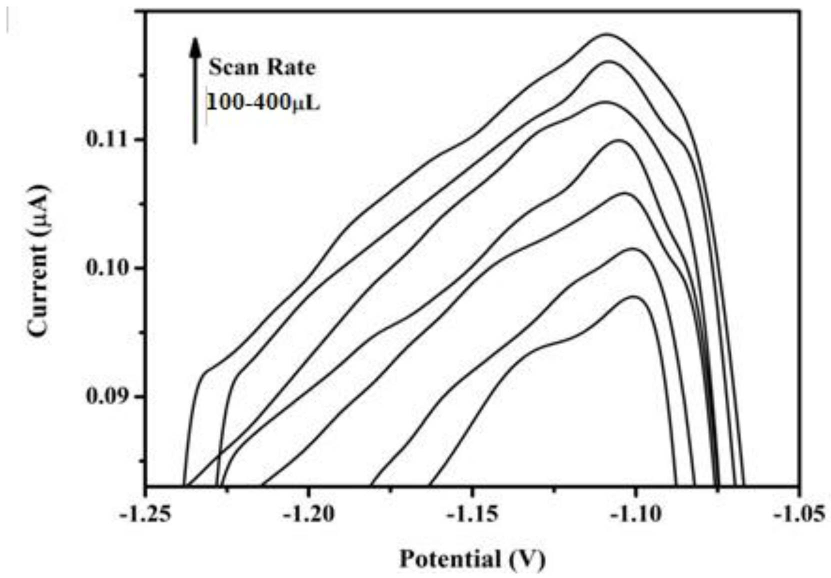


Fig:12(c): SWV of CHL on 30% (w/w) PIN/WC /SSPE

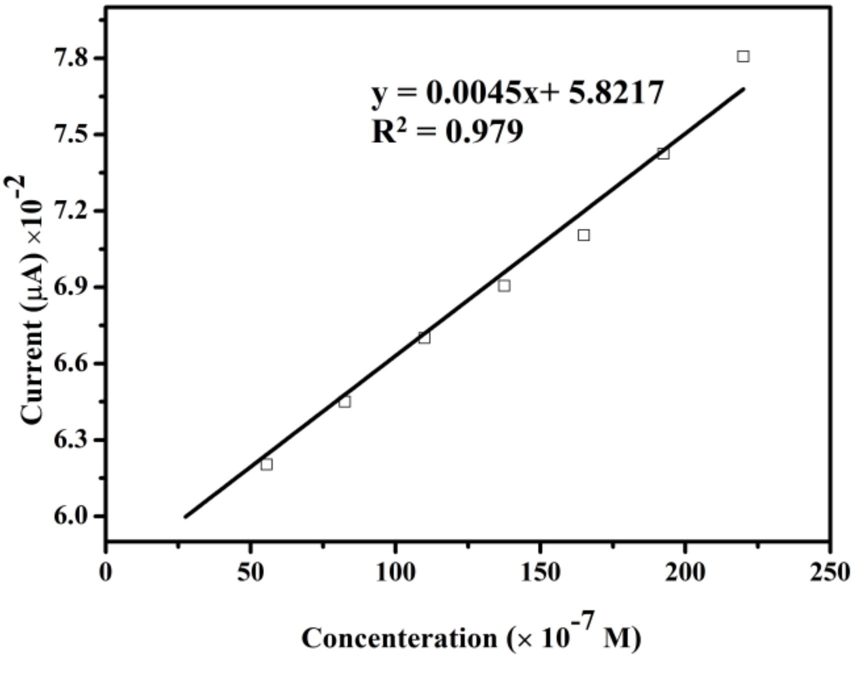


Fig 13(a): Calibration curve for quantification of CHL over bare SSPE

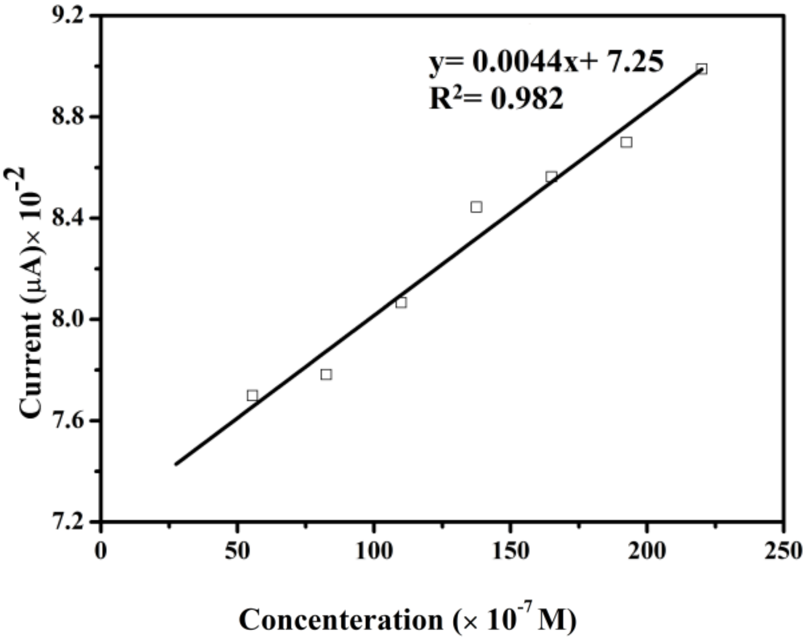


Fig 13(b): Calibration curve for quantification of CHL over 5% (w/w) PIN/WC/ SSPE

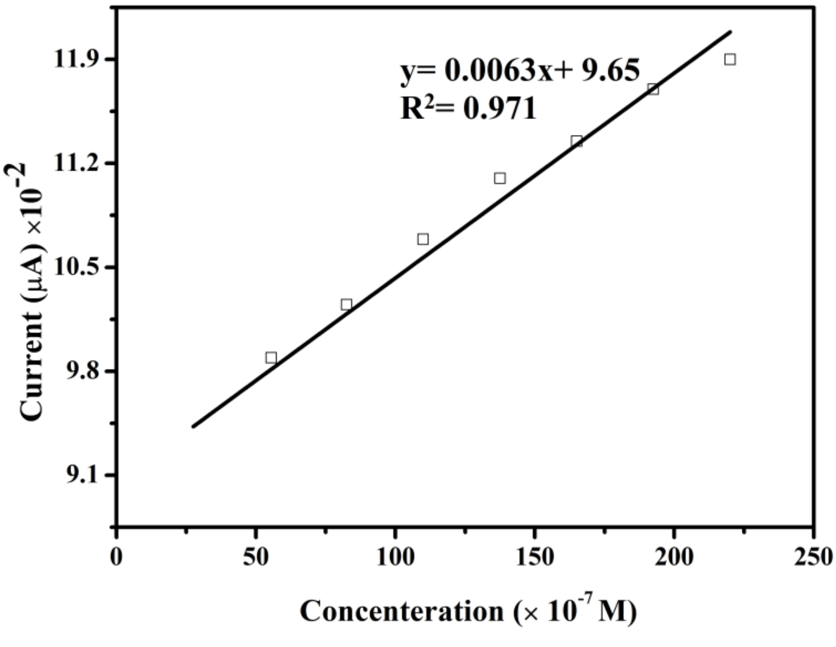


Fig13(c): Calibration curve for quantification of CHL over 30% (w/w) PIN/WC

4.4.d. Electrochemical Impedance Spectroscopy

Nyquist plot was used to study the stability of EIS spectra. The impedance data has been expressed in Hz. The spectrum was examined by equivalent electrical circuit model. The elements of the circuit are general electrical components which includes polarisation resistance or charge transfer, solution resistance and a double layer capacitor. EIS measurement is managed by a FRA connected to a potentiostat. In Nyquist plot electron transfer resistance (Ret) at higher and lower frequency range is shown by a semicircle and straight line respectively Mostly high impedance is observed at a metal surface fabricated with even and undisturbed coating which is represented by a semicircular loop whereas opening of loop represents destruction of coating (**Gong *et al.,* 2011)**. For bare electrode Rp was 8.506×10^2. The |Z| appeared at 1.200×10^3. For 5% PIN/WC composite Rp was 4.973×10^2. The |Z| appeared at 6.396×10^2. For 30% PIN/WC composite Rp was 2.669×10^2. The |Z| appeared at 2.998×10^2 . With the fabrication of SPE by PIN/WC NC, the diameter of semicircle decreased which shows that PIN/WC possesses high electrical conductivity with low charge transfer resistance. Compared with the EIS of bare GCE, the diameter of the semicircles was successively decreased in the order from 5% PIN/WC to 30% PIN/WC which indicates increase in electron transfer. This shows that 30% shows highest charge storage. Due to electrolytic diffusion opening of semi circle has been observed in 5% PIN/WC/SSPE.

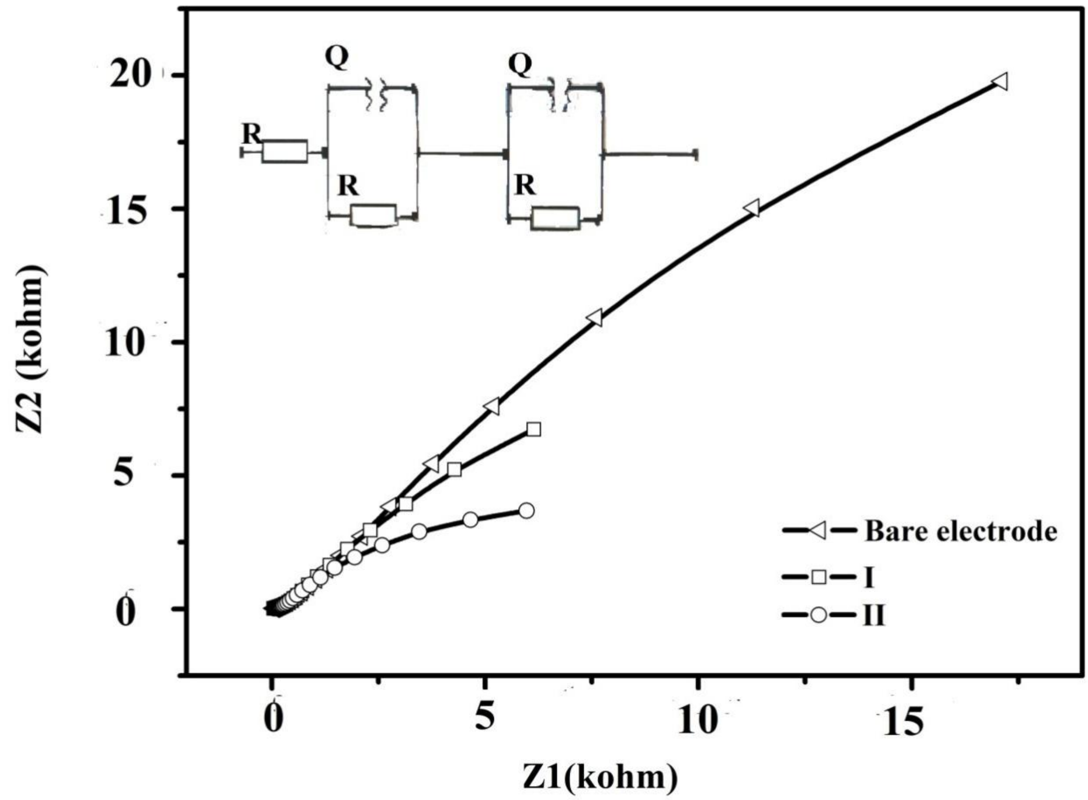


Fig:14 EIS plot of bare, 5% PIN/WC/SSPE(I) and 30% PIN/WC/SSPE (II)

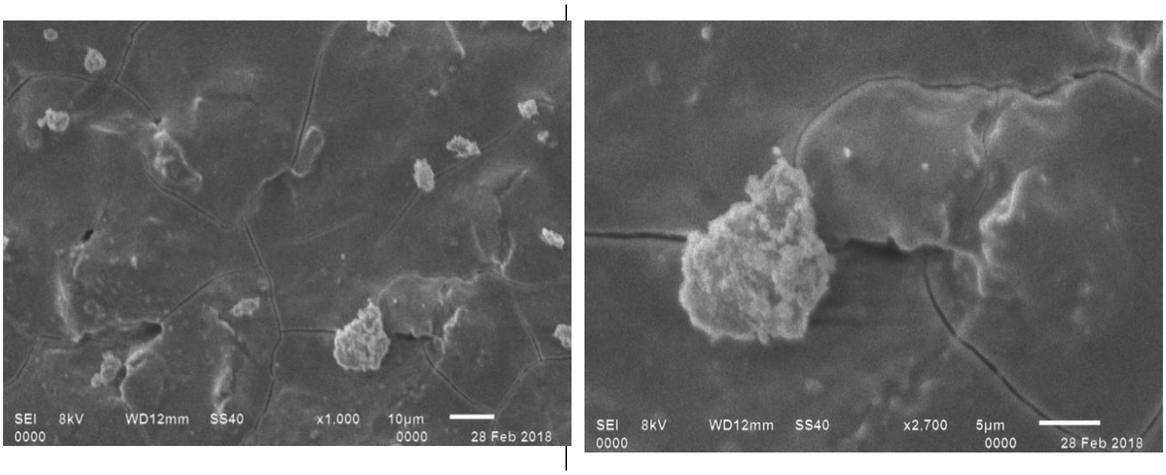


Fig 15: SEM images of 5% (w/w) PIN/WC nanocomposite deposited on SSPE (a) At 1.0 KX (b) At 2.7 KX

Summary and Conclusion

- In this study a highly sensitive and low cost PIN/WC/SSPE was developed and used for the EC detection of CHL.
- The developed PIN/WC/SSPE were characterized and analyzed by SEM and CV.
- The EC estimation of CHL was carried out by SWV and stability of prepared WE was checked by EIS.
- The LOD obtained for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE were 4.8×10^{-8} $molL^{-1}$, 5.94×10^{-8} $molL^{-1}$ and 4.49×10^{-8} $molL^{-1}$ respectively.
- LOQ for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE are 14.5×10^{-8} $molL^{-1}$, 18×10^{-8} $molL^{-1}$ and 13.6×10^{-8} $molL^{-1}$ were respectively.
- The presence of WC NPs increases the sensing current of CHL, which further increases the sensitivity of the proposed EC sensor.
- The correlation between concentration and peak current was found to be in direct relation, which shows good linearity in calibration curve.
- The designed PIN/WC/SSPE sensor has potential for the estimation of CHL in real samples.

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ABSTRACT

CHL is an OP insecticide and known to generate toxic effects by inhibiting the AChE activity. The overuse or misuse of CHL is contributing adversely to the health of living organisms and also disturb ecosystem, hence it is very important issue to detect low level amount of CHL. In the present investigations, we developed simple, selective and sensitive technique for a CHL monitoring. PIN/WC/SSPE EC sensor was designed by different proportions of PIN/WC on SSPE and characterized using SEM, CV, SWV and EIS. A well defined peak was obtained for the oxidation and reduction of CHL in CV. The LOD obtained by SWV for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE were 4.8×10^{-8} M, 5.94×10^{-8} M and 4.49×10^{-8} M respectively. LOQ for bare, 5% PIN/WC/SSPE and 30% PIN/WC/SSPE were 14.5×10^{-8} M, 18×10^{-8} M and 13.6×10^{-8} M respectively. EC investigation shows peak current was linearly related to the concentration of CHL, thus increasing detection sensitivity of proposed sensor. This could be achieved due to the synergistic blend of WC in PIN matrix having high electrical conductivity, fast electron transfer, large surface area and good absorption of NC on to the SSPE. The future prospects of developed EC sensor involves trace level CHL detection in biological and environmental samples.

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सारांश

सीएचएल एक ओपी कीटनाशक है तथा एसीएचई गतिविधि को अवरूद्ध करके जहरीले प्रभाव उत्पन्न करने के लिए जाना जाता है। सीएचएल का अत्याधिक उपयोग या दुरूउपयोग जीवित जीवों के स्वास्थ्य के प्रतिकूल योगदान एवं पारिस्थितिक तंत्र में विघन करते है। इसलिए सीएचएल की निम्न स्तर की मात्रा का पता लगाना बहुत महत्वपूर्ण विषय है। वर्तमान शोध में सीएचएल के परीक्षण के लिए सरल, चयनात्मक और संवदेनशील तकनीक विकसित किये गये हैं। पॉलीइन्डोल (PIN)/टंगस्टन कार्बइड (WC)/ स्टेनलेस स्टीलप्लेट इलेक्ट्रोड (SSPE) विद्युत रसायनिक सेंसर को एसएसपीई पर पीआईएन/डब्ल्यूसी के विभिन्न अनुपात द्वारा योजनाबद्ध किया गया था और स्कैनिंग इलेक्ट्रॉन माइक्रोस्कॉपी (SEM), चक्रीय वोल्टामेट्री (CV), वेग तरंग वोल्टामेट्री (SWV), विद्युत प्रभाव स्पेक्ट्रा (EIS) का उपयोग करके निस्रपण किया गया। CV ऑक्सीकरण और अपचयन को एक स्पष्ट शिखर द्वारा दर्शाता हैं। एसडब्ल्यूवी की सहायता से एसएसपीई 5 प्रतिशत पीआईएन/डब्ल्यूसी, 30 प्रतिशत पीआईएन/डब्ल्यूसी की परीक्षण की सीमा कमशः 4.8×10^{-8}, $molL^{-1}$, 5.9×10^{-8}, $molL^{-1}$, और 4.49×10^{-8}, $molL^{-1}$, ज्ञात हुई तथा मात्रा की सीमा कमशः 14.5×10^{-8}, $molL^{-1}$, 18×10^{-8}, $molL^{-1}$, 13.6×10^{-8}, $molL^{-1}$, ईसी परीक्षण से पता चलता है कि प्रवाह शिखर सीएचएल की सांद्रता से रेखीय समानुपातित हैं, इस कारण सेंसर की परीक्षण सीमा में वृद्वि होती हैं। यह वृद्वि पीआईएन मेट्रीक्स में डब्ल्यूसी के मिश्रण, इलैक्ट्रॉन हस्तांरण, विशाल सतह क्षेत्र और एसएसपीई पर नैनो कम्पोजिट के अच्छे अवशोषण के कारण प्राप्त होती है। भविष्य में इस निर्माणित ईसी सेंसर का उपयोग जैविक एवं पर्यावरण नमूनों में सीएचएल की न्यूनतम मात्रा के निरीक्षण के लिए किया जा सकता है।

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

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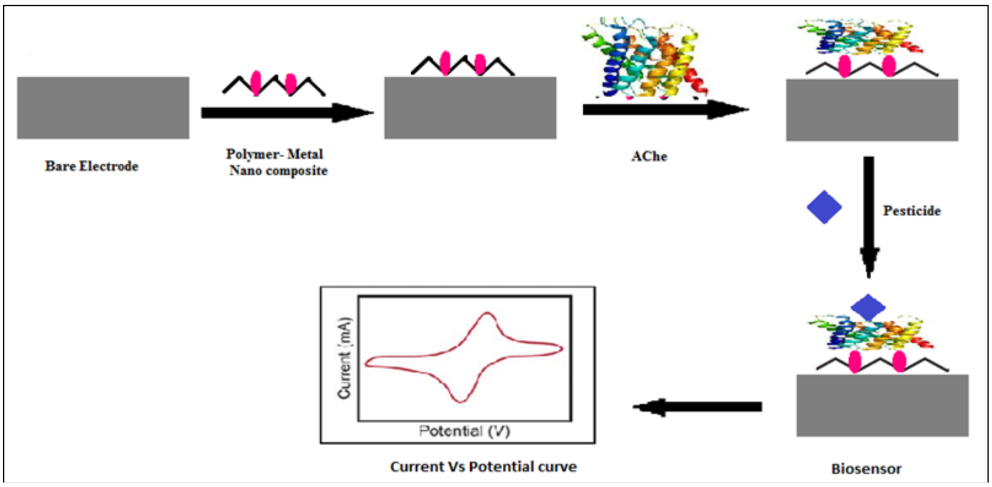
Abstract

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 ($C_7H_{14}NO_5P$) 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 assosciated 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 assosciated 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 x 10^{-8} and 1.0 x 10^{-5} molL^{-1}, and the L.O.D. was 1.0 x 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 concenteration 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 cabaryl and carbofuran was 7.5 µgL^{-1} and 4.2 µgL^{-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 (ATCl) and could catalyze the hydrolysis of ATCl. 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].

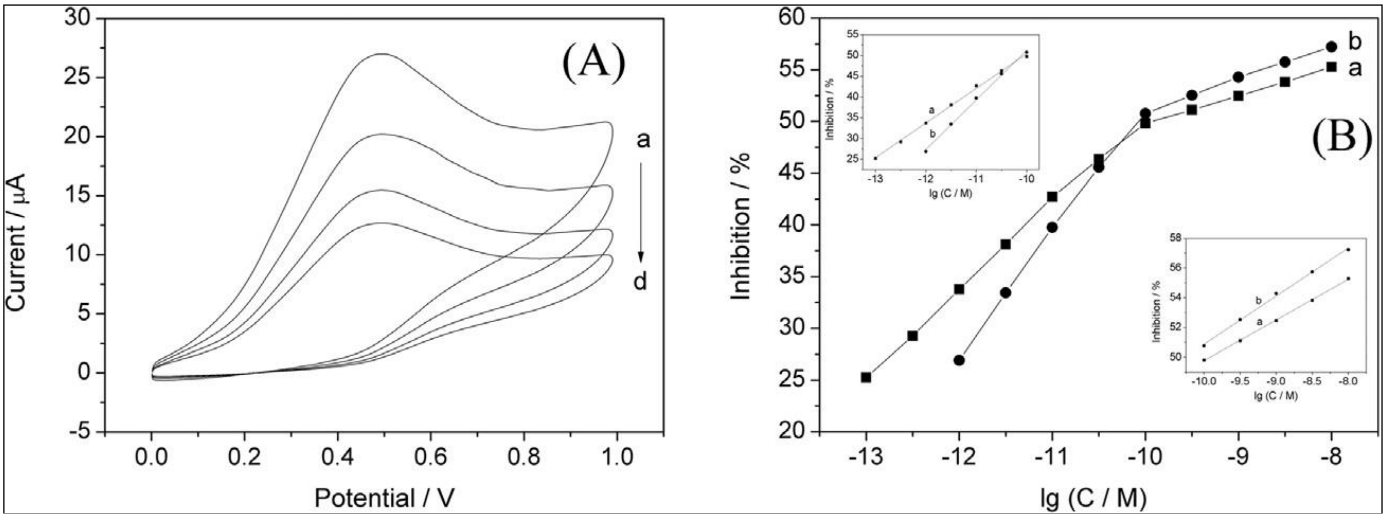


Fig 1: (A) CV of the NF/AChE-CS/PtNPs-CGR-NF/GCE in pH 7.4 PBS containing 0.5 mmolL^{-1} ATCl after incubation with 0 (a) 10^{-13} molL^{-1} (b) 10^{-11} molL^{-1} (c) 10^{-9} molL^{-1} (d) methyl parathion for 6 min. (B) Inhibition curves of NF/AChE–CS/PtNPs–CGR–NF/GCE biosensor for methyl parathion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

5.0×10^{-14} $molL^{-1}$ 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 μgL^{-1} and linearity range 0.05 to $1.0 \times 10^3 \mu gL^{-1}$ [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 µL 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 $molL^{-1}$ H_2SO_4 solution containing 5.0 $mmolL^{-1}$ $HAuCl_4$. 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 6.0×10^{-5} $molL^{-1}$, with a detection limit of 1.0×10^{-7} $molL^{-1}$. 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*-phenylinediamine) and poly(3-hydroxylthiophene), 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].

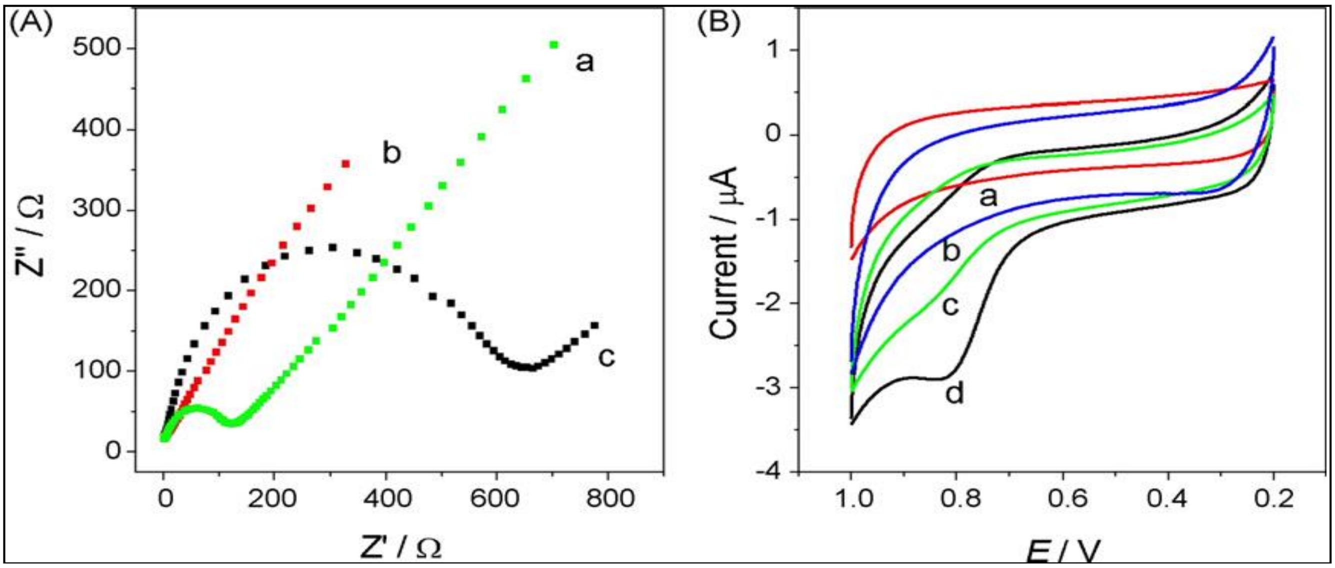


Fig 2: (A) Electrochemical impedance spectra of bare GCE (a), PAn-PPy-MWCNTs/GCE (b) and AChE-PAn-PPy-MWCNTs/GCE (c) in 5mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$. (B) Cyclicvoltammograms of bare GCE (a) and AChE-PAn-PPy-MWCNTs/GCE (b) in pH 7.0 PBS; AChE-PAn-PPy/GCE without MWCNTs (c) and AChE-PAn-PPy-MWCNTs/GCE (d) in pH 7.0 PBS containing 0.3 $mmolL^{-1}$ ATCl. 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 $nmoL^{-1}$ 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 isoproturon, 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 $molL^{-1}$ NaCl) was obtained when methiocarb in 0.1 $molL^{-1}$ H_2SO_4 was electrochemically characterized, which was used for its quantification. LDR was between 6.7×10^{-6} and 2.6×10^{-4} $molL^{-1}$ and a detection limit of 2.0×10^{-6} $molL^{-1}$. 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	SnO2	AChE	$0.05\text{-}1\times10^{3}$ μgL^{-1}	$0.05\mu gL^{-1}$	[34]
3.	Paraoxon	Amperometry	Adsorption		AuNPs	Graphene Oxide nano sheets		ND	10^{-13}	[51]
4.	Monocrotophos	Amperometry	Covalent binding		AuNPs	QDS		4.5×10^{-9}-4.5×10^{-6}	1.3×10^{-9}	[52]
5.	Methyl Parathion and Acephate	Amperometry	Encapsulation	CPE	Silicasol-gel film			3.7×10-4-1.8×10^{3}	3×10-4 $\mu molL^{-1}$	[53]
6.	Dimethylvinphos and Naftalofos	SWV and CV		GCE	Chitosan	REDUCED graphene oxide		$0.50\text{-}30\mu gL^{-1}$	For DMV-$0.036mgL^{-1}$ For Naftalotos-$0.044\ mgL^{-1}$	[54]
7.	Dichlorvos	DPV	Adsorption	SPE	Sol gel matrix	Al_2O_3		0.1-80µM	0.01 µM	[55]
8.	Paraoxon	Amperometric		SPE	Sol gel	Zinc oxide		0.127-5.010 µM	0.127µM	[56]
9.	Monocrotophos	Electrochemical	Hydrogen bonds	GCE	SiSG	AuNPs		NR	0.44 µM	[57]
10.	Chlorpyrifos-ethyl oxon	Amperometric	Encapsulation	Carbon electrode		Alumina		0.5 µM	2.5×10^{-6} µM	[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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