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Advancing Nanomaterials for Detection of Organophosphorus Nerve Agents in Forensic Application: A Review

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Abstract

The pesticide poisoning cases are one of the most common referred for examination in Forensic Laboratories. Currently, these laboratories detect pesticides in visceral samples by using conventional techniques namely Thin-layer Chromatography, Gas Chromatography, High-Performance Liquid Chromatography and Gas Chromatography–Mass Spectrometry techniques in antemortem and postmortem crime exhibits. However, these techniques are constrained by various factors including low sensitivity, efficiency, time consumption, high cost and require trained personnel to operate. The rapid development in the field of nanotechnology and nanomaterials will certainly pave the way for the development of new methods for detection of Organophosphorus pesticides in exhibits referred for forensic examination with the potentials to overcome the limitations of the conventional techniques. The researchers of various disciplines including chemical, biological, physical, medicine, explosive, fingerprinting, environment and biochemistry are exploring the Nanomaterials and Nanotechniques. Various Nanomaterials based sensors and detection techniques have been developed which have been found powerful tools for pesticides detection at very low level in food samples and contaminated water. These Nanotechniques and Nanomaterials may render great help in detection of pesticides in poisoning cases. Therefore, in forensic examination, it is decisive to apply the novel promising Nanomaterials and Nanotechniques for detection of OrganoPhosphorus (OP) pesticides with high sensitivity, selectively and rapidity.

This review paper provides an insight of using nanomaterials based techniques for detection of OP pesticides

Keywords: Organophosphorus pesticides • Acetylcholinesterase • Nanomaterials • Sensors • Forensic

Introduction

Organo Phosphorus (OP) pesticides are extensively used throughout the world in farming to protect crops from pests and simultaneously have caused serious environmental concerns as these compounds are highly toxic causing health hazards including asthma, birth defects, Alzheimer's Disease (AD), neurodegenerative diseases and in extreme cases deaths [1-3]. Organophosphorus compounds having phosphonate ester bonds are most toxic chemicals and hence use as Chemical warfare agents [4]. Recently, the political assassinations are being carried out by using Novichok. In the year 2018, Russian dissident Sergei Skripal and his daughter Yulia were attacked by deadly OP nerve agents [5]. In August 2020, in Navaley poisong case, the German Military laboratory had identified an organophosphorus nerve agent (of Novichok group) in his blood samples collected at time of admission in Hospital [6].

OP pesticides are derivatives of phosphorous and cause disruption in the normal function of body by covalently blocking the active serine site of Acetyl Cholin Esterase (AChE) enzyme. Inhibition of AChE results into excessive accumulation of Acetyl Choline (ACh) in the nervous system resultantly strong cholinergic symptoms are observed in individuals with the manifestation of muscarinic effects (salivation, urination, diarrhea, GI upset, emesis, miosis, bradycardia, bronchospasm, bronchorrhea and excess lacrimation), nicotinic symptoms (muscle twitching, contraction, asthenia and diaphragmatic breakdown) and effects in the central nervous system (agitation, confusion, lack of muscle control, trembling, convulsion, coma, respiratory paralysis and death) [7-9]. The detection of OP pesticides in biological samples is of great significance in clinical and forensic toxicology. Hence,development of detection techniques is an essential assignment for laboratories [10]. OP pesticides are usually analyzed by conventional chromatographic based techniques however recent research in applications of nanotechnology and nanomaterials has tremendously boosted up the development of new techniques [11,12]. Nanowire/nanotube, nanosheets and quantum dots of nanoscale dimension (1 nm-100 nm) possess the distinct physical and chemical properties and are utilized in the fabrication of nanosensors of Nanotechniques. The peroxidase or oxidase activities of Fe_3O_4, CeO_2, SWCNT, graphene oxide, gold, silver, palladium, platinum nanoparticles and Nanocomposites are being utilized in the biological analysis and OP pesticide detections [13-15]. Functionalized Carbon Nano Tubes (CNT), Zirconia Nano Particles (NPs) and Quantum Dots (QDs)-Nanomaterials based portable analytical systems have been developed for the on-site detection of Organophosphorus pesticides and chemical nerve agents in biological matrices [16]. These Nanotechniques have the vast potential to fulfill the forensic requirements by being economical, rapid and viable; produce accurate results and more sensitive approach over the existing conventional techniques. This review focuses on how Nano-based techniques can be used in an effective way for the detection of OP pesticides and nerve agents at trace level in gastric lavage, blood samples and viscera in forensic examination.

Nanomaterials Based Techniques for the Detection of OP Pesticides

Various nanomaterials based techniques including colorimetric assays, Electrochemical techniques including enzyme inhibition based methods, Fluorescence Based Detection Techniques (FBDT), Surface Enhanced Raman Spectroscopy (SERS) and Microfluidics based techniques for detection of pesticides have been explored and illustrated beneath (Figure 1).

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Colorimetric assays

The colorimetric techniques record the incident of color formation by shifting of absorption band in the visible electromagnetic region. The formation of color depends upon various factors including size, shape, capping agents, refractive index and aggregation properties of nanoparticles. The characteristic size-dependent optical property of gold NanoParticles (AuNPs) is recently utilized in colorimetric assays for detection of varieties of analyte [17]. The change in color properties of AuNPs were used for the first time in colorimetric assay detecting OP pesticides based upon inhibition of AChE enzyme. The blockage of AChE enzyme by paraoxon did not show any spectral changes. Furthermore, another method of OP detection was developed by observing the phenomena of Ag deposition on AuNPs surface [18,19]. A citrate-capped silver nanoparticle (AgNPs) is used for detection of dipterex using UV–Vis spectrophotometer. The dipterex concentration range was linear at 0.25 ng mL^{-1}-37.5 ng mL^{-1} corresponding to the absorbance ratio A396 nm/A520 nm with a detection limit of 0.18 ng/mL [20]. A new colorimetric method utilizing citrate-coated AuNPs was developed for speedy analysis of Methamidophos in vegetables. The absorbance at 522 nm of AuNPs was directly proportional to the concentration of Methamidophos ranging from 0.02 µg/m 1.42 µg/mL with detection limit of 1.40 ng/mL [21]. Acephate and methyl-paraoxon were detected using magnetic Fe_3O_4NPs and bienzymatic AChE and ChO combination. The detection limit of Sarin, methyl-paraoxon and acephate were 1 nm, 10 nm and 5 µm respectively [22]. Using AuNPs, Chlorpyrifos and Malathion were detected upto a concentration limit of 20 and 100 ppb in surface waters by UV Visible Spectrophotometer [23]. AuNPs, sodium chloride salt and sodium citrate were utilized for the determination of pesticide in various water samples [24].

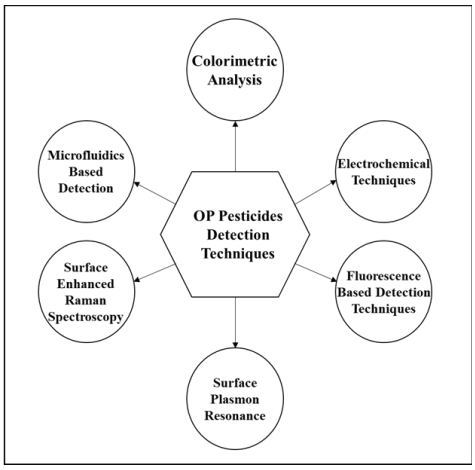

Figure 1. Schematic representation of the various nanomaterials based techniques used for OP pesticide detection.

Electrochemical techniques

Electrochemical techniques involve chemically modified electrodes which possess the unique properties of catalytic, electronic, optical, good conductivity, large surface area and miniaturization. These techniques are simple, have fast response, highly sensitive, selective and provide calibration stability. Carbon Nano Tubes (CNT), graphene, metal nanoparticles (AuNMS, PtNMS etc.), nanostructures metal oxides (ZrO_2, Fe_2O_3, etc), Nano-structured conducting polymers, quantum dots and composites nanomaterials have been widely used in the electrode modification. An electrochemical sensor was fabricated by electrodepositing the Zirconia Nano Particles (ZrO_2NPs) over the polycrystalline gold electrode using cyclic voltammetry and showed strong affinity for the phosphoric group and detecting OP pesticides and nerve agents through Square-Wave Voltammetry (SWV) [25]. The biomimetic sensor detected paraoxon-ethyl, fenitrothion and chlorpyrifos amperometrically ranging from 1.0 µmol L^{-1} and 10.0 µmol L^{-1} with a limit of detection of 0.36 µmol L^{-1}, 0.61 µmol L^{-1}, and 0.83 µmol L^{-1} respectively [26]. The biosensing platform made of a glassy carbon electrode coated with a bionanocomposite of chitosan,Carbon Nano Tube (CNTs), Hydroxy Apatite (HA) and Organophosphorus hydrolase detected paraoxon as low as 0.1 µmol L^{-1} within linear concentration range extended upto 80 µmol L^{-1} alongwith sensitivity as high as 5.10 nal $µmol^{-1}$ [27]. Lipase@ZIF-8/chitosan/Glassy Carbon Electrode (GCE) and lipase amine-modified ZIF-8(An-ZIF-8)/chitosan/GCE biosensors were used to detect methyl parathion and detection limit was found as low as 0.28 µm [28]. GCE/3DGH-AuNPs/APO electrochemical sensor was constructed for detection of Organophosphorus nerve agent mimic (DCNP (diethylcyanophosphonate) using a 3-dimensional graphene-gold nanoparticles (3DGH-AuNPs) composites. A linear relationship was obtained in the range from 1×10^{-11} to 7×10^{-8} m along with a low detection limit of 3.45×10^{-12} m [29].

Organophosphorus pesticides are also detected by electrochemical techniques by recording inhibition of AChE enzyme activity by OP pesticides (Figure 2) [30-35]. The enzymatic Nano-biosensors are fabricated by immobilizing the enzymes by entrapping, microencapsulation, covalent bonding, and cross-linkage adsorption method or by sol gel technology. A flow injection Amperometric biosensor was developed using a self-assembled AChE layer on a CNT modified Glassy Carbon (GC) electrode for monitoring paraoxon upto a limit of 0.4 pm within 6-min of incubation time [36]. An Amperometric biosensor constructed by co-immobilizing AChE and ChO enzymes on the surface of a dialysis membrane with carbon paste electrode customized with ferrophthalocyanine detected Paraoxon and Carbofuran upto 10^{-10} m [37]. Chlorpyrifos, Fenitrothion and Methyl parathion were measured upto 0.05 µm using a disposable AChE/ChO/MWCNT Amperometric screen printed biosensor [38]. An Amperometric biosensor made of rat brain AChE/(ZnSNPs) and poly (indole-5-carboxylic acid)/Au electrode detected Malathion and chlorpyrifos in spiked water samples in the range of 0.1 nm-50 nm and 1.5 nm-40 nm [39]. AChE (extracted from maize seedlings)/Iron oxide nanoparticles (Fe_3O_4 NP)/MWCNT/Au biosensor detected Malathion, chlorpyrifos, monocrotophos and endosulfan in the concentrations range of 0.1 nm-40 nm, 0.1 nm-50 nm, 1 nm-50 nm and 10 nm-100 nm respectively [40]. OP compounds namely paraoxon-ethyl and Sarin (a nerve agent) were detected using AChE/ChO/Au-Pt bimetallic nanoparticles/3-aminopropyl triethoxysilane GC electrode biosensor [41]. An electrochemical sensing device was developed by modifying GC electrode with nanocomposite Fe_3O_4NPs, poly (indole-5-carboxylic acid) and AChE for detecting Chlorpyrifos and Malathion within concentrations ranges between 1.5 nm to 70 nm and 0.1 nm to 60 nm [42]. Monocrotophos was detected by a new flow injection enzymatic electrode biosensor made of AChE/chitosan microspheres/MWCNTs/AuNPs/GC in the detection range of 0.1 µm to 10 µm and detection limit of 10 nm using Fast Fourier Transform Continuous Cyclic Voltammetry (FFTCCV) [43]. Monocrotophos in blood serum within the range of 0.05 mgL^{-1} to 3.2 mgL^{-1} was detected using AuNPs modified graphite electrode with LOD 0.05 mg/L [44]. Acetylcholinesterase (AChE)/Amino functionalized carbon nanotubes (CNTs-NH2)/AgNPs-N-F-MoS2 bio-electrode was used for electrochemical determination of Monocrotophos and Chlorpyrifos within the detection limit of 0.05 $pgmL^{-1}$ (0.2 pm) and 1 $pgmL^{-1}$ (3 pm) at a SN ratio of 3 [45]. Acetylcholinesterase/AuNRs@MS/Chitosan/TiO_2-Chitosan biosensor was employed for detection of Dichlovos (DDVP) and fenthion and gave a linear detection ranges from 0.018 µm (4.0 ppb) to 13.6 µm with a detection limit (LOD) 5.3 nm (1.2 ppb) and 1.3 nm (0.36 ppb) respectively [46].

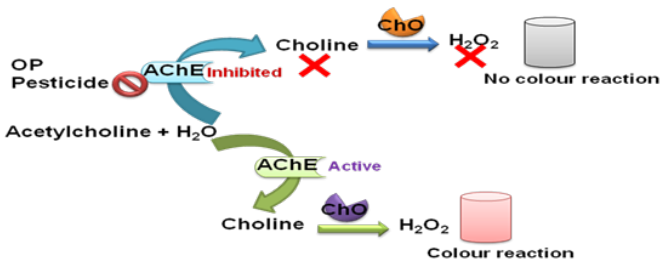

Figure 2. Graphical representation shows the working principle of enzyme inhibition based pesticide detection.

Immunological methods using nanomaterials are also reported for detection of OP pesticides. Phosphorylated AChE, a potential biomarker of exposure to Organophosphate (OP) pesticides was detected within the linear range of 10 pm to 4 nm and detection limit upto 8.0 pm using Zirconia Nanoparticles (ZrNPs) and quantum dots (ZnS@CdS, QDs)-based immunosensor [47].

Fluorescence Based Detection Techniques (FBDT)

Fluorescent Based Detection Techniques (FBDT) is based on the principle of quenching and surface modified fluorescence phenomenon. Semiconductor Quantum Dots (QDs) nanoparticles are brighter, reduce photo-bleaching effect, have longer lifespan making and ideal for utilizing in fluorescent sensing platform [48]. Based on Quenching phenomenon, a silicon quantum dot sensor was developed for detecting Carbaryl (a carbamate pesticide), parathion, diazinon and phorate (OP pesticides) with the detection limit of 7.25 ng/L, 32.5 ng/L, 67.6 ng/L and 0.19 mg/L respectively [49]. Acetylcholinesterase inhibitors were detected by measuring the fluorescence intensity produced by Cadmium Sulfide quantum dots (CdSQDs). The fabricated biosensors could detect parathion methyl (PM) pesticide as low as 0.05 ppm. The dynamic range for the pesticide detection was observed from 0.05 ppm to 1 ppm [50]. A Dimethyl-Dichlorovinyl Phosphate (DDVP) was detected by monitoring the activity of AChE using fluorimetric substrate gold nanoclusters stabilized by Bovine Serum Albumin (BSA) [51]. An upconversion Fluorescence(FL) biosensor detected diazinon in agricultural samples using Acetylcholinesterase(AChE) modulated FL "off-on-off" strategy and a linear detection range from 0.1 ng/mL to 50 ng/mL was observed with LOD 0.05 ng/mL [52]. OP pesticides were detected using AuNPs by Surface Enhanced Fluorescence spectroscopy (NSEFs). The 1 µm of OP pesticide induces fluorescence by releasing Eu3+ ions from the AuNPs surface [53].

Surface Plasmon Resonance (SPR)

A Surface Plasma Resonance (SPR) phenomenon occurs when the incident light is targeted on the nanomaterials and stimulating the resonance oscillation of electrons at the interface [54]. By using this technique, various pesticides residues have been detected in test samples. The optical changes were recorded when the test sample flowed over the surface of gold/silver sensor. Portable Surface Plasmon Resonance (SPR) immunosensor using gold-coated sensing surface detected chlorpyrifos in real water samples at 55 ng L^{-1} with detection limits ranged from 45 ng L^{-1} to 64 ng L^{-1} [55]. AChE modified Localized Surface Plasmon Resonance (LSPR) using gold nanoparticles detected paraoxon in the range of 1-100 ppb with a detection limit 0.234 ppb [56]. An intense signal was observed when the chlorpyrifos-imprinted Fe_3O_4@PDANPs interacted with the AChE immobilized SPR sensor chip and detected chlorpyrifos concentration ranging from 0.001 µm to 10 µm with a detection limit of 0.76 nm [57].

Surface Enhanced Raman Spectroscopy (SERS)

Surface Enhanced Raman Spectroscopy is surface-sensitive techniques which enhances the Raman scattering effect of molecules adsorbed by nanomaterials and has provided a scope for OP pesticides detection. Malathion and Melamine were detected using SERS–active substrate made of AgNPs trapped filter membrane [58]. SERS technique was used to detect chlorpyrifos residue in apples using AuNPs [59]. Five OP pesticides including methidathion were separated and identified from tea leaves samples using thin layer chromatography (TLC) and SERS [60]. Silver nanodendrites (AgNDs) provided a high Raman enhancement factor for SERS analysis and were used to detect 0.1 ppm concentrations of Pyridaben on vegetables, tea and fruits [61].

Microfluidics based detection

Microfluidic technique uses miniaturized devices which have potential for rapid analysis with liquid flow control, minimal reagent consumption and strong interdisciplinary uses [62]. A Microfluidic system can perform a complete biochemical/chemical analysis using small sample and a lesser amount of reagent [63]. Using Microfluidic chip, OP pesticides residue of Paraoxon, methyl parathion and fenitrothion in water were detected within detection limits 0.21 µg/mL, 0.4 µg/mL, 1.06 µg/mL in less than 140s [64]. An Acetylcholine esterase (AChE)-Choline oxidase (ChOx) bienzymatic-modified biosensing micro-system and the ferrocenyl electron shuttle were used to detect Organophosphorus pesticides [65]. A 3D-Polydimethylsiloxane (PDMS) Microfluidic passive micromixer channel was used for detection of methyl parathion [66]. A photometric detection of OP pesticide in seawater was reported by using Microfluidic chip and the results were found comparable to chromatographic techniques with nanoliter reagent consumption and a correlation coefficient of 0.963 [67]. The Quantum dots(QDs)-Acetylcholinesterase enzyme(AChE) aerogel based fluorescence Microfluidic sensor detected OPs in spiked fruit samples within detection limit of 0.38 pm and detection range from 10^{-5} m to 10^{-12} m [68].

A comparison of various nanomaterials based analytical techniques for pesticide detection is summarized in Table 1.

Table 1. Comparison of various nanomaterials based analytical methods for pesticide detection.

S.No	Techniques	Nanomaterials	Detection limit	Linear range	Incubation time	Pesticides used	Refer-ence
1.				**Colourimetric**			
i.		Citrate-capped silver nanoparticles	0.18 ng/mL	0.25 ng mL^{-1}–37.5 ng mL^{-1}	15 min	Dipterex	[16]
ii.		Citrate-coated Au nanoparticles	1.40ng/mL	0.02 µg/mL–1.42 µg/mL	30 min	Methamidophos	[17]
iii.		Fe_3O_4 magnetic nanoparticle (MNP)	10 nm and 5 µM	NR	15 min	Methyl-paraoxon Acephate	[18]
iv.		Gold nanoparticles	20 ppb and 100 ppb	20 ppb-125 ppb 100 ppb-250 ppb	2 min	Chlorpyrifos Malathion	[19]
v.		Cerium oxide nanoparticles	8.62 ppb 26.73 ppb	0 µg/mL-0.7 µg/mL 0 µg/mL-0.2 µg/mL	NR	Dichlorvos methyl-paraoxon	[20]
2.				**Electrochemical**			
i.		CNTs, hydroxyl-apatite (HA)	0.1 µmol L^{-1}	80 µmol L^{-1}	NR	Paraoxon	[27]
ii.		GCE/3DGH-AuNPs/APO	3.45 M × 10^{-12} M	1 × 10^{-11} M to 7 × 10^{-8} M	NR	DCNP(diethyl-cyanophosphonate)	[29]
iii.		ZnS and poly (indole-5-carboxylic acid)	0.1 nm and 1.5 nm	0.1 nm–50 nm and 1.5 nm–40 nm	10 min	Malathion and chlorpyrifos	[36]

iv.	Fe_3O_4/ c MWCNT	0.1nm, 0.1nm 1 nm, 10 nm	0.1 nm–40 nm, 0.1 nm–50 nm, 1 nm–50 nm 10 nm–100 nm	10 min	Malathion, chlorpyrifos, Monocrotophos endosulfan	[37]
v.	(Fe_3O_4NPs/ Pin5COOH)	0.1 nm 1.5 nm	0.1 nm to 60 nm 1.5 nm to 70 nm	10 min	Malathion Chlorpyrifos	[42]
vi.	AuNPs and MWCNTs	10 nm	0.1 µm to 10 µm	1 min	Monocrotophos	[43]
vii.	AuNPs	0.05 mg/L	0.05 mgL^{-1} to 3.2 mgL^{-1}	5 min	Monocrotophos	[41]
viii.	CNTs-NH2)/ AgNPs-N-F-MoS2	0.05 $pgmL^{-1}$ 1 $pgmL^{-1}$	NR	NR	Monocrotophos Chlorpyrifos	[45]
ix.	AuNRs@MS/ Chitosan/TiO_2-Chitosan Chitosan	5.3 nm and 1.3 nm	0.018 µm to 13.6 µm	NR	Dichlorvos Fenthion	[46]
3.			**Fluorescence**			
i.	A silicon quantum dot	7.25 ng/L, 32.5 ng/L, 67.6 ng/L 0.19 mg/L	NR	NR	Carbaryl, parathion, diazinon phorate	[46]
ii.	CdSe and CdTe quantum dots	0.05 ppm.	0.05 ppm to 1 ppm	5 min	Parathion methyl	[47]
iii.	Gold nanoclusters	13.67 pm	0.032 nm to 20 nm 1.	60 min	DDVP	[51]
iv.	NaGdF4: Yb/ Tm UCNPs	0.05ng/ mL	0.1 to 50 ng/mL	NR	Diazinon	[52]
4.			**SPR**			
i.	Gold-coated sensing surface	55 ng L^{-1}	45 ng L^{-1} to 64 ng L^{-1}	20 min	Chlorpyrifos	[55]
ii.	Gold nanoparticles with AChE	0.234 ppb	1 ppb-100 ppb	NR	Paraoxon	[56]
iii.	Fe_3O_4 with polydopamine nanoparticles	0.76 nm	0.001 to 10 µm	30 min	Chlorpyrifos	[57]
5.			**SERS**			
i.	Silver nanoparticles	6.3 ppb	12.6 ppm and 6.3 ppb 12.3 ppm to 61.5 ppb	NR	Melamine Malathion	[55]
ii.	Gold nanoparticles	0.13 mg/kg	0.13 mg/kg to 7.59 mg/kg	NR	Chlorpyrifos	[56]
iii.	Colloidal silver nanoparticles	0.1 ppm	0.1 ppm to 10 ppm	NR	Methidathion	[57]
6.			**Microfluidics**			
	Silver nanoparticles	0.21 µg/mL, 0.4 µg/ mL, 1.06 µg/mL	240, 98, and 62 nA/mM	less than 140s	Paraoxon, methyl parathion and fenitrothion	[64]
	Silver nanoparticles	0.1 ppm	2×10^{-5} mol/mL to 10×10^{-5} mol/mL	NR	Methyl parathion	[63]

Discussion

In forensic laboratories, conventional chromatographic methods are mainly applied for the separation and detection of OP pesticides in visceral samples. However, these techniques are expensive, time consuming, require trained personnel, need sample pretreatment, laboratory equipments and are unsuitable for outdoor applications. In order to keep pace with increasing demands of food and other related industries, nanomaterials based techniques/technologies are being used for detection of pesticides. These techniques are highly sensitivity, inexpensive, simple to operate, requires minimum samples, suitable for indoor and outdoor analysis and are serving as an excellent complementary to the classical analytical techniques. Hence, for better utilization, there is a strong need to apply these techniques for trace residue analysis in forensic examination. In this review, progresses in the field of nanotechnology and Nanotechniques for the detection of OP compounds are being enumerated. The enzymatic or non-enzymatic colorimetric assays for the detection of pesticides are compiled. The non-enzymatic electrochemical electrodes/sensors are successfully fabricated and utilized for OP pesticides detection. The AChE enzyme based biosensors have been fabricated with functional Nano-scaffolds of novel nanomaterials and enzymes to detect the pesticides in trace level. The simple potentiometer, voltammetry and Amperometric techniques are being used for biosensing purpose. The fluorescent techniques using quantum dots, SPR and SERS have appeared the new branches of analytical techniques for detection of pesticides. TLC-SERS technique is applied for OP detection. The miniaturized devices made by hyphenation of Micro-fluidics and Nanotechniques have further opened a new horizon for detection of pesticides as these offer better sensitivity, suitability for trace analysis, easy operation and minimum solvents consumption.

Conclusions and Future Perspectives

The conventional techniques can be used for OP pesticide detection nevertheless the advancement in Nanoscience and Nanotechnology may be utilized for developing novel detection techniques. Acetylcholinesterase based biosensors and other hyphenated techniques would serve as alternative techniques for better detection of pesticides in comparison to the existing expensive chromatographic methods. Therefore, it is of utmost important that Nanotechnology based techniques/sensors must be fabricated for detection of specific OP compounds in biological samples.

By using new approaches of Nanotechnology in forensic examination, parameters including sensitivity, real time detection, response time, solvent consumption, trace analysis and selectivity can be improved. Work on the examination of Organophosphorus pesticides in forensic samples using the above Nanotechnology based techniques is under exploration with great promise for future applications.

Conflict of Interest

The authors declare that there is no conflict of interest.

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