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Review Article

NANOMATERIALS BASED ACETYLCHOLINESTERASE BIOSENSORS FOR ORGANOPHOSPHORUS COMPOUNDS DETECTION: REVIEW

VIKAS DHULL[1], NEERAJ DILBAGHI[1], VIKAS HOODA[2]*

[1]Department of Bio & Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar 125001, India, [2]Centre for Biotechnology, Maharshi Dayanand University, Rohtak 124001, India.
Email: advance.biotech@gmail.com

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ABSTRACT

Due to intense pressure on agriculture for supporting exponentially growing population pesticides are used on an alarming scale. As these pesticides contain Organophosphorus (OP) compounds which are highly toxic and interfere with functioning of enzyme Acetylcholinestrase (AChE) and finally affecting Central Nervous System (CNS). So, there is an urgent need to monitor OP compounds concentration regularly in the marketed food products and even in the environment (water, soil). Here we focus on the different nanomaterials used for the fabrication of the AChE biosensors for detection of OP compounds which is based on inhibition of AChE. The merits and demerits of the different nanomaterials which are being used as supports are also discussed. The mode of detection, detection limit, linearity range, time of incubation, storage stability of the biosensors is also reviewed. Nanomaterial as an important class of supports used for the AChE biosensors due to their valuable properties. Among all the nanomaterials used Gold nanoparticles (AuNPs) have gained an advantage as they are explored with time.

Keywords: Pesticides, Acetylcholinesterase, Acetylcholinestrase Biosensor, Immobilization support, Detection Limit.

INTRODUCTION

Pesticides are a key part of agriculture which is in regular use worldwide for enhancing productivity to meet the demands of remarkably growing population. These have insecticidal property due to which they are excessive use [1, 2]. But their regular use is harmful for human health and the surroundings due to presence of organophosphorus (OP) compounds. These OP compounds accumulate in fruits, vegetables, grains and contaminate water [3, 4]. Their concentration is increasing in an environment with the high rate. Organophosphorus (OP) constitute an important classes of toxic compounds that lead to irritation in eyes, abdominal pain, convulsions, respiratory system failure and other neurological disorders [5-10]. As per Environmental Protection Agency (EPA) organophosphates are highly toxic to wildlife and humans [1].

The OP pesticides are irreversible inhibitors of enzyme AChE by attachment of hydroxyl group on active site with serine of enzyme acetylcholinestrase (AChE, EC 3.1.1.7) which is a key enzyme for proper functioning of central nervous system (CNS) of the humans. This inhibition of AChE enzyme, results in accumulation of acetylcholine (ACh) neurotransmitter in body which further interferes with response of muscles resulting in respiratory related problems, myocardial malfunctioning and finally death [11,12]. The toxicology of these OP pesticides depends on their chemical structure [12, 13]. Regulatory aspects and the guideline levels are there for permissible residues in drinking water [14]. There is a need to develop new protocols for detection of OP compounds in various samples which are selective and sensitive [15].

Analytical methods are there for monitoring the concentration of these toxic compounds in fruits, vegetables etc. The analytical methods used for this purpose include capillary electrophoresis [16], colorimetry [17], gas chromatography (GC) [18], mass spectrometry (MS) [19], thin layer chromatography [20, 21] and high performance liquid chromatography (HPLC) [22]. Immuno-assays are also effective as they are highly selectivity, sensitivity and reproducibility, but suffer from the drawback as they require corresponding antibodies [23, 24]. Sample preparation is also required which is a complex process, time consuming, requires expensive equipments which is not present in all laboratories. Biosensor overcomes the problems associated with analytical methods. Biosensors are fast, sensitive, and reliable for detection of traces of OP compounds.

In this present article, the nanomaterials based biosensors which work on the inhibition principle of enzyme AChE are reviewed in order to monitor the presence of these toxic compounds in different samples.

Nano structured materials for the biosensing

Nanostructured materials are basically known for their fundamental property of being quantum sized and having the large surface area. These properties of nanomaterials make them more advantageous than that of other bulky materials which are being used as a support for immobilization of enzymes. The nanostructures are characterized as Zero Dimensional (0D) which includes nanoparticles and quantum dots, 1 Dimensional (1D) such as carbon nanotubes (SWCNTs/MWCNTs) and nanowires & finally 2 Dimensional (2D) having graphene sheets and metallic platelets in this orientation category [25] (fig. 1). These nanostructures are explored more and more because of their characteristics to improve biosensors with respect to their electrical conductivity, surface area, detection limit, easy to synthesize in laboratory, controlling & maintain their size etc.

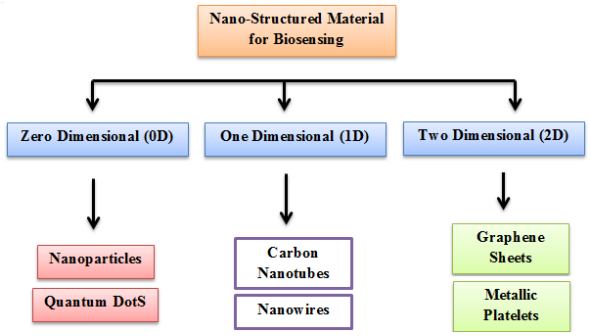

Fig. 1: Characterization of Nanomaterials

Nanostructured materials for OP compound sensing

The nanomaterial biosensors are based on the catalysis by organophosphorus hydrolase (OPH) on OP compounds (fig. 2) and

on the other hand inhibition effects of OPs on activity of AChE. When inhibitor (OP) is not present the substrate acetylthiocholine is converted into thiocholine and acetic acid (fig. 3). The catalytic activity of AChE for the hydrolysis reaction of acetylcholine to thiocholine is drastically inhibited by trace amounts of OPs present in the environment (fig. 4). Nanomaterial-based AChE electrochemical sensors have been extensively applied for AChE activity assay and OPs screening by using different supports and transducer material [26], surface plasmon resonance (SPR) [27], absorption [28] and fluorescence spectroscopy [29,30]. By using nanomaterials such as carbon nanotubes, Au nanoparticles (AuNPs), ZrO2 nanoparticles, quantum dots (QDs) as immobilization support matrices, there is a dramatic increase in the electrocatalytic activity with very high sensitivity, selectivity and accuracy for detection of OP compounds [31]. The localized SPR (LSPR) property of AuNPs covalently coupled with AChE has been exploited for OPs determination [27]. Layer-by-Layer (LBL) method has been used for integrating CdTe QDs with AChE which resulted in a highly sensitive fluorescence biosensor for detection of OPs in vegetables and fruits based on enzyme inhibition mechanism [29]. Large variety of OP compounds has been hydrolysed by OPH which produce less toxic products such as p-nitrophenol and diethyl phosphate. Several types of OPH-based nano-biosensors have been introduced recently, including fluorescence [32-35] and amperometric biosensors [36].

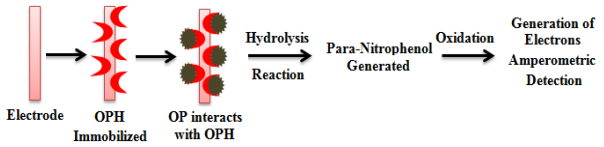

Fig. 2: Basic reaction involved in OPH biosensors

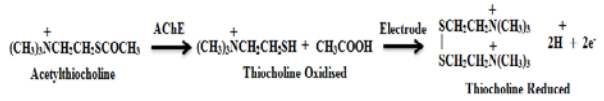

Fig. 3: Reaction in absence of inhibitor (OP Compound)

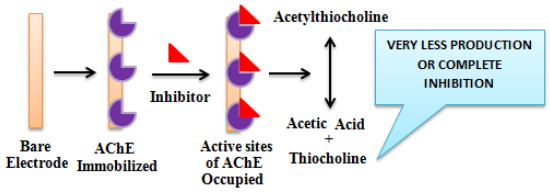

Fig. 4: Reaction in presence of inhibitor

Nanomaterial based OPH biosensor

The OPH biosensors are used for the detection of paraoxon, methyl parathion, parathion, coumaphos etc. OPH hydrolyse the OP compounds resulting in change of pH of the solution which can be measured. Basic scheme involved showing in fig. 2. This change in pH is due to the breaking of the bonds such as P-CN, P-O, P-S [37] etc. Nanoparticles are proving to be a boom in the field of biosensing due to their invaluable properties such as large surface area, are highly conductive, good catalytic property etc. the rate of electron transfer is enhanced to a great extent and also the nanomaterial possesses high affinity towards the enzyme OPH. They can be synthesized in the laboratory and even their particle size can be adjusted according to the need. OPH based biosensors are developed using SWCNTs [38-39], MWCNTs [40,41], AuNPs [42].

Nanoparticles based Ach E biosensors

Metallic nanoparticles are unique with respect to their electronic and electrocatalytic properties which depend upon the size and morphological structure [43, 44]. These metallic nanoparticles possess high mechanical strength; they are biocompatible, for conducting oxygen ion and retaining biological activity [45]. Nanoparticle (NP)-based Ach E biosensors have many advantages both in terms of stability and in terms of enhancing the catalytic reduction of redox species. A variety of nanoparticles including silver, platinum, palladium, cooper, cobalt and others have been explored for fabrication of the working electrode in biosensor development [46-50]. But, gold nanoparticles (AuNPs) are explored and exploited to a greater extent as a fabrication material for biosensors development because of its valuable capability to enhance electronic signal when biological components are in contact with working electrode fabricated with nanoparticles Organic stabilizers are also in use to synthesize nanomaterials of different morphologies depending upon the need.

Dendrimers are tridimensional organic macromolecules with highly defined functional structure [51]. These dendrimeric structures stabilize and maintain the integrity of metallic nanoparticles that was reported by Crooks and co-workers [52]. As an example, polyamidoamine dendrimers (PAMAM) were used as template for nanoparticles synthesis or for nanoparticles nucleation in nano reactors. Functional groups are also present on dendrimers is also an important subject of studies in the field of nanostructured thin films fabrication and also, in development of hybrids with metallic nanoparticles.

One such approach was reported recently in which hybrids of PAMAM-AuNPs in multilayer thin films based on LBL technique were used to enhance the charge transfer in modified working electrodes leading to electroactive nanostructured membranes (ENM) concept [53]. Different supports used for immobilization of enzyme (Table 1): PAN/gold nanoparticles (AuNPs) onto Pt electrode [54], GnPs/Chitosan/GCE [55], AuNPs–CaCO3 bioconjugate/Au electrode,

Table 1: Nanoparticles based supports used for Ach E immobilization.

Mode of detection	Transducer	Enzyme immobilization method	Minimum detection limit	linearity	Substrate/enzyme inhibitor	Time of incubation (min)	Storage stability (days)	Reference
Amperometric	PAN/AuNPs/Pt electrode	Covalent	0.026×10-5 µM	3.6×10-7-3.6×10-4 µM	Paraoxon	20	30	54
Voltammetric	GnPs/Chitosan/GCE	Covalent bonding	1.58×10−10 M	NR	Cholropyrifos	10	10	55
Amperometric	AuNPs/PB/GCE	Adsorption	3.5×10-9 µM	4.48×10-3-4.48× 10-2 µM	Monocrotophos	10	30	56
Amperometric	ZrO2/CHIT composite film/GCE	Adsorption	1.3, 5.0×10-3 and 1.7 µM	6.6-440, 0.01-0.59 and 8.6-520 µM	Phoxin, Malathion and imethoate	15	30	57
Amperometric	Gold-platinum bimetallic NPs/GCE	Crosslinking with glutaraldehyde	50×10-4, 40×10-3 and 40 µM	50-200×10-3, 1.40-50×10-3 and 40-60 µM	Paraoxon ethyl, sarin and aldicarb	25	NR	58
Amperometric	AuNPs/GCE	Adsorption	7.0×10-3 µM	28×10-3-170×10-3 µM	Methamidophos	10	7	59
Amperometric	PB and CHIT/GCE	Glutaraldehyde crosslinking	0.113×10-4, 0.703×10-4,	0.45×10-4-0.045, 0.234×10-3-	Paraoxon and chlorpyrifos-ethyl	10	NR	60

			0.19410-4 and 0.3310-4 μM	0.046, 0.116×10-3-0.0194 and 0.167×10-3-0.0335 μM	oxon			
Fourier transform continuous cylclic voltammetry	MWCNTs/AuNPs-CHIT/GCE	Adsorption	0.01 μM	0.1-10 μM	Monocrotophos	NR	50	61
Amperometric	CdS-decorated garphene nanocomposite	Adsorption	3.4×10-3 μM	9.9×10-3-9.93 μM	Carbaryl	2	20	62
Amperometric	CHIT-GNPs/Au electrode	Chemisorption/esorption	0.1×10-3 μM	0.3×10-3-60.5×10-3 μM	Malathion	15	NR	63
Amperometric	AuNPs/Au electrode	Adsorption	33×10-3 μM	10×10-3-135×10-3 μM	Carbofuran	20	7	64
Amperometric/Flow injection analysis system	PbO2/TiO2/Ti	Adsorption	0.1×10-3 μM	0.01-20 μM	Trichlorfon	10	5	65
Amperometric	PB-CHIT/GCE	Covalent	3.0×10-3 μM	0.01-0.4 and 1.0-5.0 μM	Carbaryl	10	30	66
Amperometric	Er-GRO/Nafion	Adsorption	2.0 ng mL−1	5.0-100 ng mL−1 and 1.0-20 ng mL−1	Dicholrvos	10	28	67
Amperometric	Au–PtNPs/3-APTES/GC electrode.	Cross linking	150–200 nM, 40–50 nM, and 40–60μM for Paraoxon ethyl, sarin, and aldicarb	NR	Paraoxon ethyl, sarin, and aldicarb	10	NR	68
Amperometric	PAN-AuNPs	Covalent immobilisation	7.39×10−11 g L−1	10−10-10−7 g L−1	paraoxon	NR	20	69
Voltammetric	CdTe-GNPs film.	Covalent binding	0.3 ngmL−1	1-1000 ngmL−1 and 2-15ngmL−1	monocrotophos	8	30	70
Amperometric	SiSG-AuNPs	Surface adsorption	0.6 ng/ml	0.001-1 μg/ml and 2-15 μg/ml	monocrotophos	10	30	71

AuNPs/PB/GCE [56], ZrO2/CHIT composite film/GCE [57], gold–platinum bimetallic NPs/GCE [58], AuNPs/GCE [59], PB and CHIT/GCE [60], calcium carbonate–CHIT composite film/GCE [61], CdS-decorated graphene nanocomposite [62], CHIT–GNPs/Au electrode [63], AuNPs/gold electrode [64], PbO2/TiO2/Ti [65], PB–CHIT/GCE [66], Er-GRO/Nafion [67], Au-PtNPs/3-APTEs/GCE [68],, PAN-AuNPs [69], CdTe AuNPs Film [70], SiSG-AuNPs [71].

Quantum dot as immobilization support for Ach E

Quantum dots are the luminescent fluorophores. These are semiconductor particles having dimensions confined to the nanometre scale [72]. They are very important candidate for the fabrication of variety of biosensors as they possess size dependent properties and are dimensionally similar to biological molecules [73-74]. Sensitive sensors have been developed as QDs can be coupled with the variety of biological molecules. They suffer from demerits such as large size (10 to 30 nm), blinking behaviour.

The supports which are used for the immobilization of the enzymes are (Table 2): Supports used for immobilization of enzyme: CdTe QDs/AuNPs/chitosan (CHIT)/GCE [75], CdTe QDs/Au electrode [76], and poly-(allylamine hydrochloride)/CdTe QDs/glass [77], Mn: ZnSe d dots [78], CdTE QDs/Au electrode [79].

Table 2: Quantum dots used as support for Ach E immobilization

Mode of detection	Transducer	Enzyme immobilization method	Minimum detection limit	linearity	Substrate/enzyme inhibitor	Time of incubation (min)	Storage stability(days)	Reference
Electrochemical	AuNPs-SiSG/GCE	Hydrogen bonds	0.44 μM	NR	Monocrotophos	10	30	75
Amperometric	CdTe QDs/AuNPs/CHIT/GCE	covalent	1.34 μM	4.4×10-3-4.48 and 8.96-67.20 μM	Monocrotophos	8	30	76
Optical	Poly(allylamine hydrochloride)CdTe QDs/glass	Electrostatic interaction	1.05×10-5 and 4.47×10-6 μM	1.0×10-6-1.0 and 1.0-0.1 μM	Paraoxon Parathion	15	35	77
Fluorescence quenching	Mn: ZnSe d-dots	NR	1.31_10_11 mol/	4. to.84x10_11 to 4.84x10_6 mol/L	paraoxon	10	NR	78
Amperometric	CdTE QDs/Au electrode	covalent	2.98×10-3 μM	4.96×10-3-2.48 μM	Carbyl	10	30	79

CNTs and Nanowires based Ach E biosensors

Carbon nanotubes (CNTs) are the one dimensional (1D) nanomaterials which are known for their wide application in chemical and biological sensing of different analytes in variety of samples. CNTs have hollow graphitic structure of cylindrical shape having fast electron transfer rate and blessed with good electrocatalytic effect [80-82]. These magnetic nanomaterials used

in the conjugation with biological material are the basis of electrochemical biosensing. The CNTs possess unique properties related to surface area, conformation stability, high bioactivity and substrate biocatalyst interaction [83, 84]. The carbon-based electrodes such as carbon pellet [85], carbon fibers [86], carbon felt [87], carbon black slurry [88], glassy carbon [89], graphite particles and graphite [90] have been replaced by CNTs.

Different supports used are (Table.3): Multiwalled carbon nanotubes (MWCNTs)/PAN membrane onto Pt electrode [91], polyamidoamine (PAMAM)–gold/carbon nanotubes (CNTs)/GCE [92], AuNP–polypyrrole (PPy) nanowire composite film modified GCE [93], PPy and PANI copolymer doped with MWCNTs/GCE [94], SWCNT–CoPC/SPE [95], MWCNTs–gold nanocomposites/GCE [96], AuNPs–MWCNTs/GCE [97], MWCNTs–CHIT composite/GCE [98], SWCNT modified FGE [99], CNT web modified GCE [100]. Nanowire is the well-ordered polymer chain structure having small cross dimensions and with the high surface to volume ratio. PPy nanowires are one of the nanowires which have been used for the fabrication of biosensors [101]. The PPy nanowires are conducting polymers which are organic in nature. These conducting polymers act as enzyme immobilization matrix in which enzyme can be entrapped or covalently attached. As these are conducting in nature they can allow the transfer of charge through them, can be easily prepared, and are stable also [102-104]. The nanowires can be used in conjugation with the nanoparticles for the fabrication of working electrode. AuNPs and PPy were used together as an immobilization matrix for AChE in detection of OP compounds. AuNPs was electrodeposited onto the PPy nanowires for the stable immobilization of AChE enzyme leading to the development of OP biosensor for detection of methyl parathion105. Due to the combination of nanoparticles and nanowires the porosity of the matrix is increased as a result of which large effective surface area for enzyme immobilization is available, good conductivity and high catalytic activity can be achieved.

Table 3: CNTS and Nanowires as support for AChE immobilization

Mode of detection	Transducer	Enzyme immobilization method	Minimum detection limit	linearity	Substrate/enzyme inhibitor	Time of incubation (min)	Storage stability (days)	Reference
Amperometric	MWCNTs/PAN membrane/Pt electrode	Affinity bonds using concanavalin A	5.0×10-9 µM	3.6×10-8-3.6×10-5 µM	Paraoxon	20	120	91
Amperometric	PAMAM-gold/CNTs/GCE	Electrostatic interaction	4.0×10-3 µM	4.8×10-3-9.0×10-2 µM	Carbofuran	9	21	92
Electrochemical	AuNPs-PPy nanowires composite film modified GCE	Entrapment	7.5×10-3 µM	0.018-0.45 and 1.89-17.0 µM	Methyl parathion	12	30	93
Amperometric	PPY and PANI copolymer doped with MWCNTs/GCE	Adsorption	3.02×10-3 µM	0.030-1.51 and 3.027-75.67 µM	Malathion	15	30	94
Amperometric	Single-walled CNTs-CoPC/SPE	Covalent	0.01 and 6.3×10-3 µM	0.018-0.181 and 6.36×10-3-0.159 µM	Paraoxon and malaoxon	15	3	95
Amperometric	MWCNTs-Au nanocomposite/GCE	Hydrophilic surfacefor biomolecule adhesion	1.81×10-3 µM	3.0×10-3-3.027 µM	Malathion	8	30	96
	AuNPs-MWCNTs/GCE	Adsorption	1.0×10-3 µM	0.1×10-3-7.0×10-3 µM	NR	30	NR	97
Amperometric	MWCNTs-CHIT composite/GCE	Covalent	NR	NR	Carbaryl, Malathion, dimethoate and monocrotophos	8	30	98
Potentiometric	SWCNT modified FGE	Cross linking	25–35 nM and 15–20nM for Sarin and DFP respectively	20–60 nM and 20–80 nM for sarin and DFP respectively	Sarin and DFP	5	30	99
Amperometric	CNT-Web modified glassy carbon electrode	Adsorption	1nM	20–1000 nM	methyl parathion	20	NR	100

Graphene sheets and metallic platelets based Ach E biosensors

Graphene nanosheet was experimentally synthesized in 2004 in which the sp^2 hybridized carbon atoms were packed like honeycomb two dimensional sheets [106-108]. Graphene is a two dimensional carbon based nanomaterial with valuable physical, chemical and excellent electrocatalytic properties [109,110]. Nanocomposites, nanoelectronics, electrochemical resonators and ultra sensitive sensors can be synthesized using graphene nanosheet [111-114]. The high surface area of graphene sheet is favourable for immobilization of enzyme and have small band gap which is responsible for conducting the electrons with high efficiency [110], graphene based hybrid nanocomposites are also prepared; as these nanocomposites materials are functionalized they may enhance the sensing performance of the biosensor [115]. The planer geometry of graphene nanosheet makes it more advantageous over the carbon nanotubes [116]. With this, the graphene nanosheet is in more close contact with the surrounding medium rather than that of tube shaped carbon nanotubes. Graphene based chemical sensors have low electronic noise from the thermal effect which is due to high temperature of the solution or the reaction mixture provides higher sensitivity [117]. Interlocking is also shown by graphene nanosheet with the adsorbed target or the analyte [118].

The exfoliated graphite nanoplatelets (xGnPs) have emerged as a substitute of carbon nanotubes [119]. These xGnPs possess structural, electrical, mechanical and thermal properties imparted by graphite. Both graphite and carbon nanotubes are well known for their property of surface adsorption [120]. Adsorption is more in case of metallic platelets as they are arranged in sheets due to which it has the high surface area as compared to that of the hollow structure of carbon nanotubes. Presence of xGnPs on the surface of working electrode enhances the electrical conductivity and on the other hand reduces electrode fouling [121]. Some of the supports used (Table 4) are graphite–epoxy composite/SPE [122], TCNQ modified graphite [123], TiO2-decorated grapheme/GCE [124], graphite, nanoplatelet–CHIT composite/GCE [125], CdS-decorated grapheme nano composite [126].

Table 4: Graphene and Nanoplatelets used as support foe Ach E immobilization

Mode of detection	Transducer	Enzyme immobilization method	Minimum detection limit	linearity	Substrate/enzyme inhibitor	Time of incubation (min)	Storage stability (days)	Reference
Amperometric	Graphite-epoxy composite/SPE	Crosslinking	1.0×10-4 and 1.0×10-5 µM	NR	Paraoxon and carbofuran	15	5	122
Amperometric	TCNQ modified-Graphite	Screen Printing	1 ppb	0-5 x 10-3M	Methamidophos	10	NR	123
Amperometric	TiO2-decorated grapheme/GCE	Adsorption	1.4×10-3 µM	4.9-74.5 and 74.5-9.9×103 µM	Carbyl	3	20	124
Voltammetric	Graphitenanoplatelet-CHIT composite film/GCE	Covalent	1.58×10-4 µM	1×10-4-1.0 µM	Chloropyrifos	10	10	125
Amperometric	CdS-decorated garphene nanocomposite	Adsorption	3.4×10-3 µM	9.9×10-3-9.93 µM	Carbaryl	2	20	126

Table 5: Merits &Demerits of different supports

AChE Immobilization Supports	Examples	Merits	Demerits
Membrane Based	Nylon and cellulose nitrate, hybrid mesoporous silica, poly-(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) (PAN), cellophane, polyacrylamide, Biodyne and Immunodyne, Hybond N+.	Artificial membranes have high selectivity for bio-elements; possess higher degree of flexibility, mechanical durability, wider pH range for use, and higher specific activity.	Membrane fouling. The pores of semipermeable membrane are blocked leading to hindrance in the passage of solute.
Non Conducting Polymer Matrices	PVA–SbQ polymer onto screen-printed electrode (SPE), PVA–SbQ membrane/Pt electrode, and polyamidoamine (PAMAM)–gold/carbon nanotubes (CNTs)/GCE, MSF/PVA/GCE, Nylon net, PVA/AWP, CoPC modified PVA-AWP electrode.	Easily prepared in the lab. Variety of the functional groups can be generated on supports by chemical treatment. Provides microenvironment to enzyme which increases storage stability.	Acts as barrier between electron and transducer which influence sensitivity of electrode Finally working of biosensor affected.
Conducting Polymer Matrices	Mercaptobenzothiazole/polyaniline(PANI)/Au electrode, PANI/CNTs wrapped with single stranded DNA (ssDNA)/Au electrode, Silk fibronin matrix, CS/ALB/GCE, PB/GCE, GnPs/Chitosan/GCE.	Thickness of film, functionalization, conductivity etc. can be adjusted. Can also be used for the enzyme entrapment during electropolymerization and used in the uniform covering of the help of the polymer film	High cost, difficult in processing, lack of mechanical stability after doping, difficult to fabricate, short life span.
Sol Gel	Sol–gel crystals derived from tetramethyl orthosilicate (TMOS), sol–gel film on a glass cap, TMOS sol–gel film, chromoionophore (ETH5294) doped sol–gel film, Al2O3 sol–gel matrix, sol–gel matrix on 7,7,8,8-tetracyanoquinodimethane (TCNQ), AuNPs–SiSG, alumina sol–gel, bromothymol blue doped sol–gel film, zinc oxide sol–gel, and silica sol–gel film, Sol-gel/Carbon electrode.	The first and the foremost important property of the sol gel support is that the pore size can be adjusted according to the need. They are also chemically inert, do not show swelling in the aqueous medium, have photo-chemical and thermal stability. The antibodies and the enzymes can specially be immobilized. Do not allow the leakage of the enzyme in the medium.	Some of the accountable demerits includes denaturation of biomolecules take place at high acidic condition and/or high alcohol concentration, the protocols used for the sol gel film formation are not amenable for coating the curved surfaces of substrates such as optical fibers, sufficient signals require high level of biomolecules in sol gel thin films but it is not possible in case of proteins that are insoluble or aggregate in the alkoxy silane solution.
Screen Printing	TMOS sol–gel film/SPE, Al2O3 sol–gel matrix SPE, SPE (TCNQ mediator [7,7,8,8-tetracyanoquinonedimethane] in the graphite electrode), Prussian blue (PB) modified SPE, cobalt(II) phthalocyanine (CoPC)/SPE, o-phenylenediamine onto carbon/CoPC SPE, graphite–epoxy composite/SPE, SWCNT–CoPC/SPE, PET chip SPE.	Screen printing involves immobilization of the biological molecules or the biological receptor in their active form.	Screen printing is instable, high cross-sensitivity towards anion, limited life span.
Quantum Dots	CdTe QDs/AuNPs/chitosan (CHIT)/GCE, CdTe QDs/Au electrode, and poly(allylamine hydrochloride)/CdTe QDs/glass, Mn: ZnSe d dots, CdTE QDs/Au electrode.	Highly luminescent photostable fluorophore, semiconductor particles of nanometre scale, having great size dependent properties and are dimensionally similar with the biological molecules.	They suffer from demerits such as large size (10 to 30 nm), blinking behaviour if no emission interrupt longer periods of fluorescence.

CONCLUSION

Nanomaterials based biosensors have found wide range of applications for the detection of OP compounds in water, soil, food samples etc. Different fabrication strategies have been applied for the development of nano based biosensors. They provide real-time qualitative and quantative information related to the composition of samples. Sample preparation for these nano fabricated sensors is also limited. Nano sensors must be validated for their analytical performance in detection of the wide range of the toxic nerve agents present in different samples.

All the nanomaterials used have their own merits and demerits according to their utility (Table 5). AChE nano sensors fabricated with AuNPs have advantage over the other nanomaterials such as CNTs/Nanorods/Nanowires/QDs based AChE sensors. The Au nanoparticles can easily be synthesized in the laboratory and their size can be adjusted according to our need. They are highly conducting and can be used as transducer material in working electrode fabrication. It has been reported that AuNPs based sensors detect the OP compounds ranging from nM-pM concentration. The sensitivity, selectivity & reusability must be enhanced by exploring the properties of gold nanoparticles. The validation of these sensors

must be done for the *in vitro* detection of human samples such as blood, urine etc.

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CONFLICT OF INTEREST

The authors have no conflict of interest in publication of this article.

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