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Review

Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review

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ABSTRACT

Pesticides released intentionally into the environment and through various processes contaminate the environment. Although pesticides are associated with many health hazards, there is a lack of monitoring of these contaminants. Traditional chromatographic methods—high-performance liquid chromatography, capillary electrophoresis, and mass spectrometry—are effective for the analysis of pesticides in the environment but have certain limitations such as complexity, time-consuming sample preparation, and the requirement of expensive apparatus and trained persons to operate. Over the past decades, acetylcholinesterase (AChE) inhibition-based biosensors have emerged as simple, rapid, and ultra-sensitive tools for pesticide analysis in environmental monitoring, food safety, and quality control. These biosensors have the potential to complement or replace the classical analytical methods by simplifying or eliminating sample preparation and making field-testing easier and faster with significant decrease in cost per analysis. This article reviews the recent developments in AChE inhibition-based biosensors, which include various immobilization methods, different strategies for biosensor construction, the advantages and roles of various matrices used, analytical performance, and application methods for constructing AChE biosensors. These AChE biosensors exhibited detection limits and linearity in the ranges of 1.0×10^{-11} to 42.19 μM (detection limits) and 1.0×10^{-11}–1.0×10^{-2} to 74.5–9.9×10^{3} μM (linearity). These biosensors were stable for a period of 2 to 120 days. The future prospects for the development of better AChE biosensing systems are also discussed.

Pesticides (e.g., herbicides, fungicides, insecticides) are widely used in agriculture due to their high insecticidal activity [1,2]. However, the presence of pesticide residues in food, water, and soil has become a major issue in environmental chemistry [3,4]. Among the pesticides, organophosphorus (OP)[1] and carbamate insecticides form an important class of toxic compounds. Their toxicity is based on the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), which is essential for the functioning of the central nervous system (CNS) of humans and insects. This results in the accumulation of the acetylcholine (ACh) neurotransmitter, which interferes with muscular responses and causes respiratory and myocardial malfunctions and even death [5,6]. OP pesticides (Fig. 1A), by accumulating in vegetables and fruits, influence the quality of agricultural products and harm the health of consumers. The toxicity of organophosphate and carbamate pesticides varies considerably, depending on the chemical structure of the pesticide [6,7] (Fig. 1B).

The contamination of soil and food due to pesticides has caused a serious concern; therefore, to watch over the safety of marketed food supplies. international organizations (e.g., Food and Agriculture Organization) regulate their maximum residue levels on foods and agricultural commodities. Contamination of ground and surface water by pesticide residues had been regarded as transitory because the focus was on OP pesticides, which were of very low water solubility and had a strong tendency to attach to particulate matter. In addition, the soil profile is known to act as a purifying filter. However, the information accumulated during recent years about generally more soluble organochlorine pesticides and other herbicide compounds has shown the presence of pesticide residues in both surface and ground water. Consequently, policies have been made to reduce contamination of ground and surface water.

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[1] *Abbreviations used:* OP, organophosphorus; AChE, acetylcholinesterase; CNS, central nervous system; ACh, acetylcholine; CE, capillary electrophoresis; MS, mass spectrometry; GC, gas chromatography; HPLC, high-performance liquid chromatography; NAD(P)H, β-nicotinamide adenine dinucleotide (phosphate); SAM, self-assembled monolayer; PVA–SbQ, polyvinyl alcohol-bearing styrylpyridinium groups; PAN, poly-(acrylonitrile–methylmethacrylate–sodium vinylsulfonate); MWCNT, multiwalled carbon nanotube; AuNP, gold nanoparticle; MSF, mesocellular silica foam; GCE, glassy carbon electrode; SPE, screen-printed electrode; PAMAM, polyamidoamine; CNT, carbon nanotube; PEI, polyethylenimine; PANI, polyaniline; ssDNA, single-stranded DNA; PPy, polypyrrole; TMOS, tetramethyl orthosilicate; TCNQ, 7,7,8,8-tetracyanoquinodimethane; PB, Prussian blue; CoPC, cobalt(II) phthalocyanine; QD, quantum dot; CHIT, chitosan; NP, nanoparticle; ITO, indium tin oxide.

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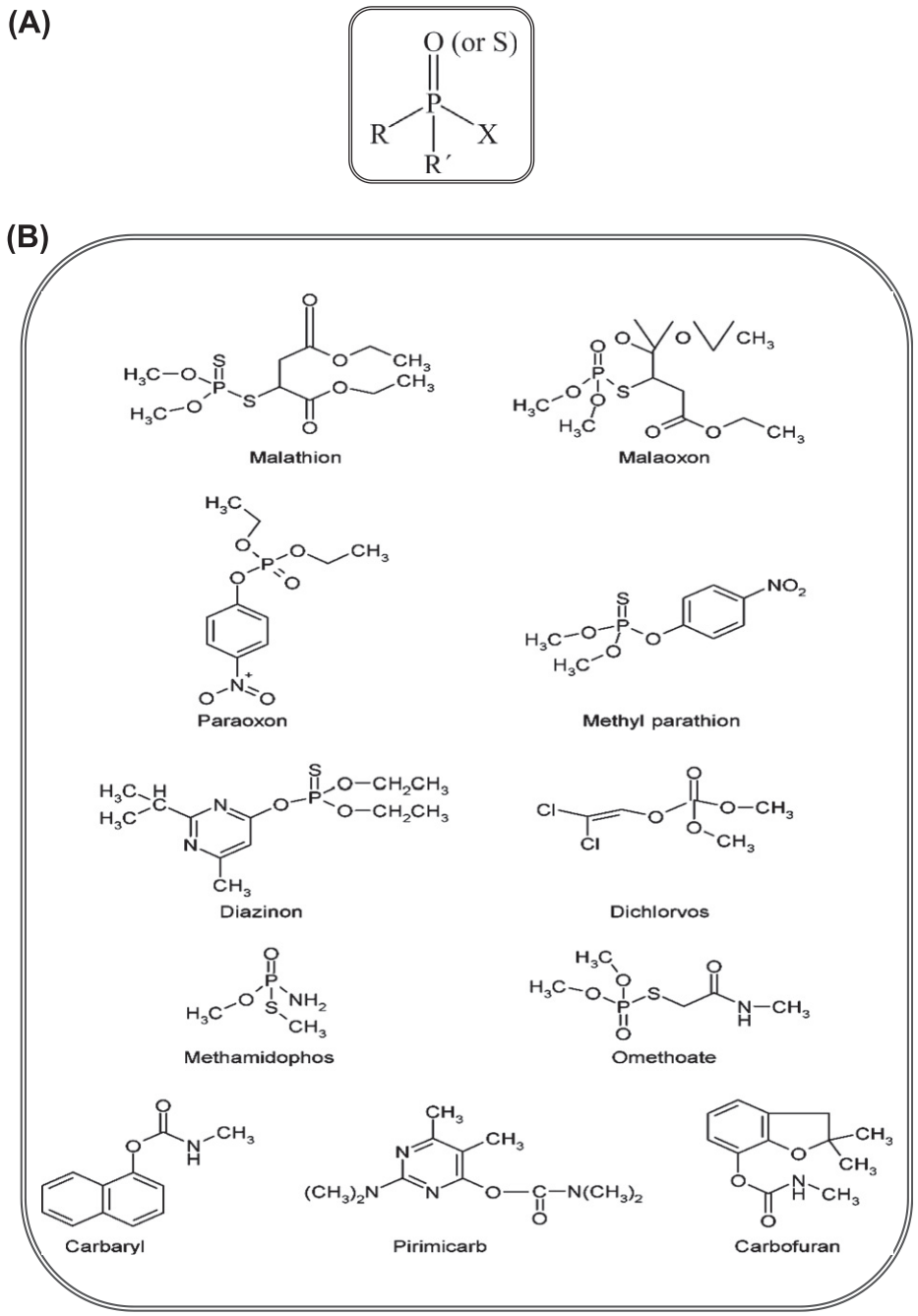

Fig.1. (A) General formula for OP compounds. (B) Structures of the main pesticides used as targets in AChE biosensors.

Regulatory limits and guideline levels have also been introduced for permissible residues in drinking water [8].

To protect human health from possible hazards, it is pertinent to develop sensitive, fast, and reliable methods for determination of OP pesticides in water, vegetables, and fruits [9]. However, analysis of pesticides in environmental, food, clinical, and forensic samples is a difficult task due to the matrix complexity and low concentrations of the target compounds. Colorimetry [10], capillary electrophoresis (CE) [11], mass spectrometry (MS) [12], gas chromatography (GC) [13], high-performance liquid chromatography (HPLC) [14], thin layer chromatography [15,16] coupled with different detectors and spectral techniques, and flow injection analysis [17] are some analytical methods that are most commonly employed to trace environmental analysis of pesticides and also are part of regulations in monitoring the environmental pollutants. Although these methods provided fruitful results, these are cumbersome and time-consuming, require sample preparation, and suffer from drawbacks such as usability only in highly specialized laboratories with very expensive equipment and trained personnel. However, biosensors overcome these limitations. Compared with various methods available for the determination of pesticides, biosensing methods provide advantages such as simplicity, rapidity, specificity, sensitivity, low cost, relatively economic equipment, and user-friendly operation (Table 1).

Here we provide an overview of the biosensing systems, since their introduction in 1993, that employed AChE inhibition by pesticides for their environmental and food analysis.

Table 1
Comparison of characteristics of traditional analytical techniques and biosensing techniques.

Traditional analytical techniques		Biosensors	
Positive	Negative	Positive	Negative
Sensitive	Time-consuming	Rapid real-time detection	Limited commercial application
Specific	Expensive	Cost-effective	
	Laboratory monitoring	Portable (in situ monitoring)	
	Trained laboratory personnel	Simple use	
	High-tech equipment	Limited sample preparation	
	Extensive sample preparation	Highly sensitive	
	More organic solvent consumption	Specific	
	Not reusable	Reusable	
		Less organic solvent consumption	

Acetylcholinesterase

ACh acts as a neurotransmitter both in the CNS and in the nerve skeletal muscle junction (Fig. 2). ACh is readily hydrolyzed to choline and acetic acid by AChE. It is mainly found at neuromuscular junctions and cholinergic synapses in the CNS, where its activity/concentration terminates synaptic transmission [18]. AChE has a very high catalytic activity; each molecule of AChE degrades approximately 25,000 molecules of ACh per second into choline and acetic acid. The produced choline is transported back into the nerve terminals to reuse it in synthesizing new ACh molecules [19]. AChE belongs to the family of hydrolases whose active site is characterized by a catalytic coordinated triad of three essential amino acids: histidine, serine, and aspartic acid [20,21]. The enzyme catalysis occurs when the triad's anionic binding site attracts the positively charged quaternary ammonium group of ACh. The serine hydroxyl group attacks and cleaves the ester after its deprotonation by a neighboring histidine group in the triad [22]. However, in the presence of an inhibitor such as an organophosphate, the nucleophilic serine hydroxyl group located at the active site is covalently bound to the phosphorus atom of the organophosphate. A similar reaction occurs with the carbonyl carbon of carbamates, and this blocking of the triad serine inactivates the enzyme [23,24]. The detection methods of organophosphate and carbamate pesticides are mostly based on the principle of inhibition of cholinesterases by pesticides [24–27].

Biosensors

Biosensors are based on enzymes and either consume oxygen (e.g., all of the oxidases), produce hydrogen peroxide (excluding oxidases that produce water), or produce (indirectly) the reduced form of β-nicotinamide adenine dinucleotide (phosphate) (NAD(P)H, e.g., dehydrogenases) during the course of the catalytic reaction on the substrate of interest [28]. The general equations of these amperometric biosensors are summarized in Fig. 3.

Basic principle of AChE biosensors

Biosensors are widely used as devices suitable for fast analysis of toxic compounds. The enzyme AChE is a biorecognition element sensitive to inhibition by organophosphates as well as carbamate pesticides, nerve agents, several natural toxins [29,30], and some drugs [31]. Hence, AChE is widely used as a potent recognition element for the construction of biosensors for pesticide detection [32,33]. Biosensors based on AChE as well as butyrylcholinesterase were first reported during the 1980s. Since then, there has been a continuous improvement of cholinesterase-based biosensors due to the gradual improvement of transducer devices and the availability of pure enzymes [34]. AChE biosensors work on inhibitory effects. When the analyte is not present in the solution, the substrate acetylthiocholine is converted into thiocholine and acetic acid. Thiocholine is oxidized by the applied voltage. In the presence of an inhibitor, conversion of acetylthiocholine is decreased or even null [35]. The principle of an electrochemical biosensor based on AChE and oxidation of thiocholine is shown in Fig. 4A.

Furthermore, the anodic oxidation current is inversely proportional to the concentration of pesticides in samples and the exposed time as well. The procedure of the preparation of AChE biosensor and pesticide detection is shown in Fig. 4B.

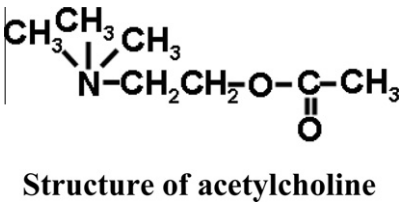

Fig.2. Structure of acetylcholine.

AChE immobilization

The most important step in the development of an enzyme sensor is the firm attachment of the enzyme onto the surface of the working electrode. This process is governed by various interactions between the enzyme and the electrode material and strongly affects the performance of the biosensor in terms of sensitivity, stability, response time, and reproducibility. There are a variety of methods by which enzymes can be immobilized, ranging from physical adsorption and entrapment to covalent chemical bonding. The different techniques used for immobilization of enzyme for construction of AChE biosensors are depicted in Fig. 5.

Physical adsorption

Physical adsorption generally consists of simple deposition of AChE onto the surface of working electrode and attachment of AChE through weak bonds such as Van der Waals forces and electrostatic interactions between the AChE and the transducer.

Merits: No damage to enzyme, simple and cheap way of immobilization, no chemical change of the support, and reversible to allow regeneration of free enzyme.
Demerits: Leakage of enzyme, nonspecific binding, overloading of the enzyme on support, short response time, poor operational and storage stability, and sensitive to changes in pH, temperature, and ionic strength [36].

Physical entrapment

Physical immobilization methods such as entrapment in sol–gel matrices and lattice of a polymer matrix or membrane have also

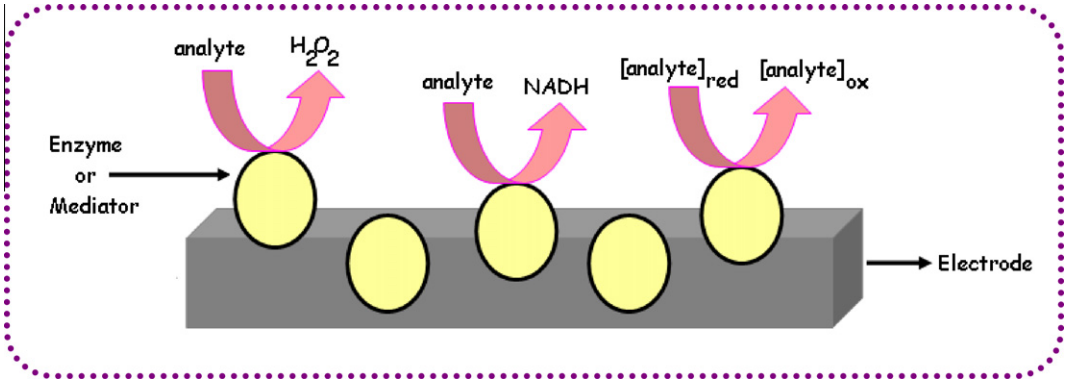

Fig.3. Schematic representation of various types of amperometric biosensors.

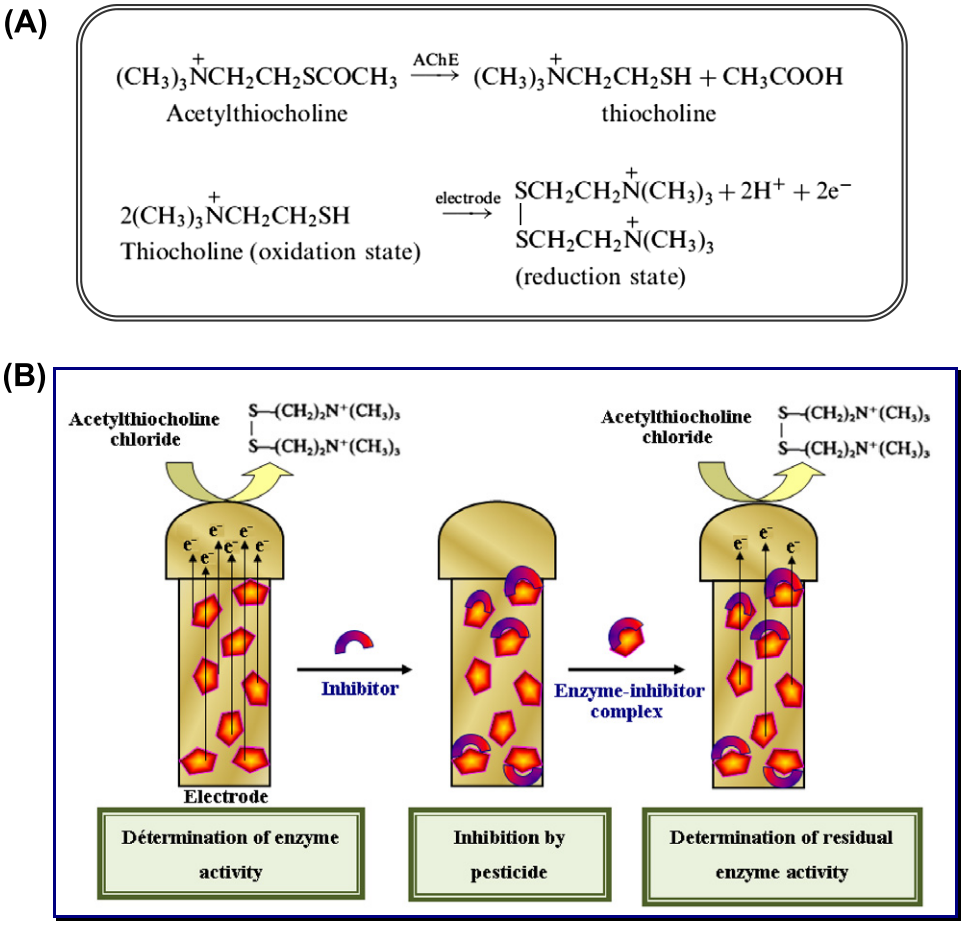

Fig.4. (A) Electrochemical reactions involved in response measurement of AChE biosensors. (B) Strategy for the measurement of immobilized AChE inhibition in pesticide solution.

been used for AChE electrodes. It has been done in such a way as to retain protein while allowing penetration of substrate.

Merits: One-step procedure at ambient or low temperature, no damage to enzyme, simple and cheap way of immobilization, no chemical change of the support, and suitable for a large variety of bioreceptors.
Demerits: Leakage of enzyme, nonspecific unstable immobilization, many biocompatible polymers available, and problems of reproducibility and control of pore size and diffusion barriers [25,37–40].

Covalent coupling

Covalent coupling of AChE is the most widely used procedure. AChE can be covalently linked to the surfaces of a transducer through formation of a stable covalent bond between functional groups of AChE and the transducer.

Merits: Absence of diffusion barriers, short response time, no enzyme leakage, and wide range of choices for selecting carrier material (making the method flexible with specific chemical and physical properties).

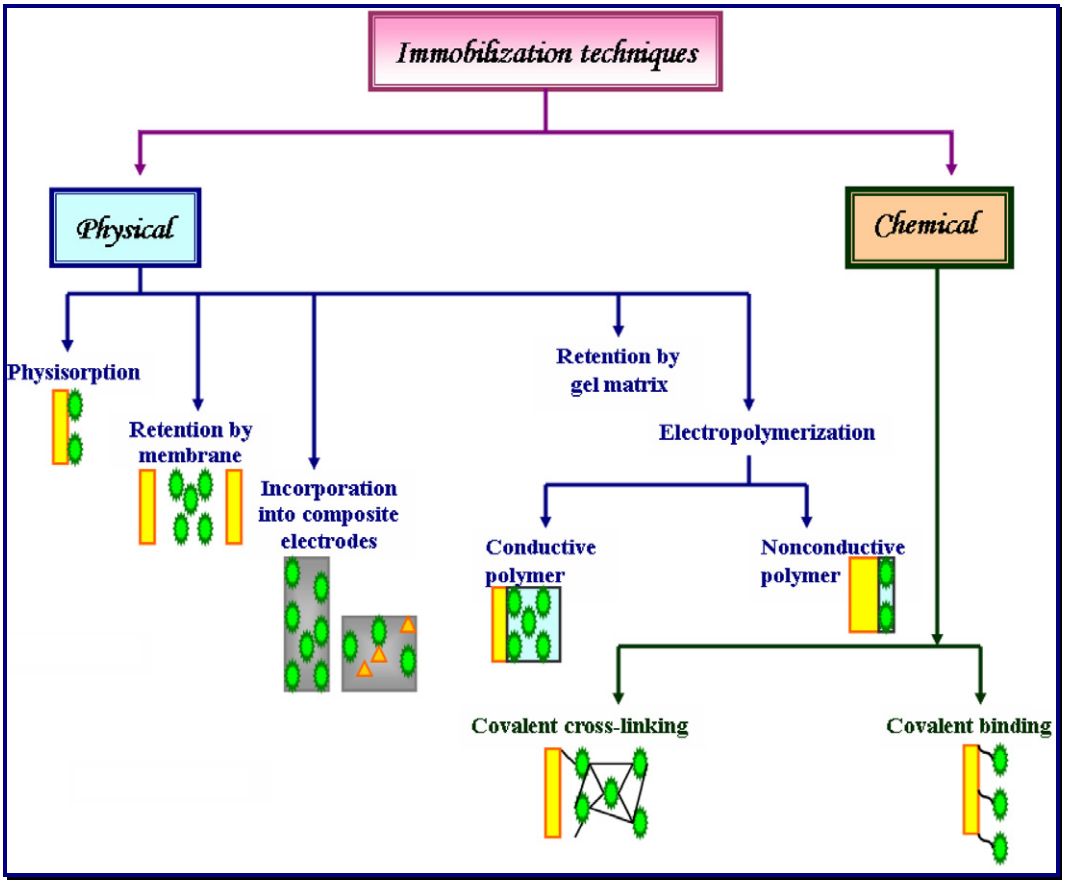

Fig.5. Immobilization techniques used for the development of ACh biosensors.

Demerits: High amount of enzyme, possible denaturation, and expensive and complicated procedures [41–45].

Self-assembled monolayer

A self-assembled monolayer (SAM) is an organized layer of amphiphilic molecules in which one end of the molecule, the "head group," shows a specific reversible affinity for a substrate. SAMs also consist of a tail with a functional group at the terminal end. Du and coworkers reported an immobilization method of AChE on cysteamine SAM modified Au electrode for carbaryl detection [46].

Merits: High degree of structural order, nanometer size, molecular recognition properties, ease of preparation, and diversity of terminal functionalities.
Demerits: Possible electrode fouling, complex, and difficult to reproduce [47,48].

Oriented immobilization

New trends focus on the development of protocols for the oriented immobilization of AChE through specific functional groups located at their surface. In this way, active sites may be faced toward the target analytes present in the sample, and substrates and products may freely diffuse in the biological layer.

Merits: Reusable surface, low amount of enzyme, and controlled and orientated immobilization [49].
Demerits: Requires the presence of specific groups in the bioreceptor molecule (e.g., histidine, biotin, concanavalin A).

Electropolymerization

The electropolymerization process involves polymerization under the influence of an electric current. Electropolymerization can be performed by holding the film at a suitable oxidation potential of the monomer or by using consecutive cyclic voltammetry in a suitable positive scan potential region.

Merits: Ability of coating electrodes, which have a small or nonuniform surface, to control the polymer thickness, prevention of interferences or electrode fouling. In addition, many polymers behave as mediators or are used for the immobilization of a mediator [50,51].
Demerits: Occurs only on conducting substrates (limiting the types of surfaces that can be electropolymerized).

Classification of AChE biosensors

Membrane-based AChE biosensors

Membrane-based AChE biosensors (based on the immobilization of enzyme on suitable matrices) offer a portable, cheap, and rapid method for the determination of pesticides. The exotic properties of biocompatible artificial membranes could make them the promising matrices for enzyme immobilization for enhancement of the sensitivity and selectivity of biosensors. The development of biosensors based on immobilized enzymes has solved several problems such as loss of enzyme (especially if expensive), maintenance of enzyme stability, and increased shelf life of the biosensor and reduction in time of the enzymatic

Table 2
AChE biosensors based on membranes for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/ Inhibitors	Incubation time (min)	Storage stability (days)	Reference
PVA–SbQ membrane/Pt electrode	Amperometric	Entrapment	7.2×10^{-5}, 1.88, and 0.049	NR	Paraoxon, maneb, and thifensulfuron methyl	30	30	[52]
Nylon and cellulose nitrate membrane/pH electrode	Potentiometric	Crosslinking with glutaraldehyde	0.038 0.077	50×10^3–2.5×10^3 50×10^3–2.5×10^3	TrichlorfonCo-Ral	15	30 15	[54]
Glass/sol–gel indicator/ polyvinylidene fluoride membrane	Fiber-optic	Crosslinking with glutaraldehyde	0.53 and 0.023	0.54–39.8 and 0.022–0.13	CarbarylDichlorvos	10	21	[55]
Poly(2-hydroxyethyl methacrylate) membrane/ oxygen electrode	DO metric	Entrapment	0.119	0.05–2.62	Aldicarb	5	2	[56]
Cellophanemembrane/AuE	Amperometric	Crosslinking	1.45	1.45–7.26	Paraoxon	15	NR	[57]
Hybrid mesoporous silica membrane/Pt electrode	Amperometric	Entrapment	1.2×10^{-3}	1.0×10^{-3}–0.3	DZN-oxon	15	80	[58]

Note. NR, not reported; DO metric, dissolved oxygen metric.

response and also offers disposable devices easily usable in stationary or flow systems.

Supports used for immobilization of AChE: Polyvinyl alcohol-bearing styrylpyridinium groups (PVA–SbQ) membrane [52], polyacrylamide membrane [53], nylon and cellulose nitrate membrane [54], glass/sol–gel indicator/polyvinylidene fluoride membrane [55], poly(2-hydroxyethyl methacrylate) membrane [56], cellophane membrane [57], hybrid mesoporous silica membrane [58], and poly-(acrylonitrile–methylmethacrylate–sodium vinylsulfonate) (PAN) membrane [59,60].
Merits: Artificial membranes have high selectivity for certain bioelements and amplify responses because of their higher degree of flexibility, mechanical durability, wider pH range for use, and higher specific activity.

Table 2 provides a comparison of analytical properties of membrane-based AChE biosensors.

Polymeric matrix-based AChE biosensors

Polymers have found wide use in the field of electronic measuring devices, especially in sensors, owing to their ability to have their chemical and physical properties tailored over a wide range of characteristics [61]. The suitably chosen polymeric matrices are found to be biocompatible, flexible, and cost-effective. Besides, these can be obtained in the form of free-standing films for the fabrication of biosensors [62].

AChE biosensors based on nonconducting polymer matrices

Supports used for immobilization of enzyme: Multiwalled carbon nanotubes (MWCNTs)/PAN membrane onto Pt electrode [59], PAN/gold nanoparticles (AuNPs) onto Pt electrode [60], mesocellular silica foam (MSF)–PVA/glassy carbon electrode (GCE) [63], PVA–SbQ polymer onto screen-printed electrode (SPE) [38], PVA–SbQ membrane/Pt electrode [52], and polyamidoamine (PAMAM)–gold/carbon nanotubes (CNTs)/GCE [64].
Merits: Easy to prepare. After an adequate chemical modification, such a polymer membrane provides various functional groups, allowing only the low-molecular-substrate molecules to reach the enzyme active centers. Nonconductive polymer membranes would provide a favorable microenvironment for the enzyme molecules that prolong the storage of enzyme.

Table 3 provides a comparison of analytical properties of amperometric nonconducting polymer-based AChE biosensors.

AChE biosensors based on conducting polymer matrices

Supports used for immobilization of enzyme: Polyacrylamide membrane/pH electrode [53], polyethylenimine (PEI)-coated GCE [65], PEI/SPE [41], mercaptobenzothiazole/polyaniline (PANI)/Au electrode [66], PANI/CNTs wrapped with single-stranded DNA (ssDNA)/Au electrode [67], AuNP–polypyrrole (PPy) nanowire composite film modified GCE [68], PPy and PANI copolymer doped with MWCNTs/GCE [69], ZnS, and poly(indole-5-carboxylic acid)/Au electrode [70].
Merits: Polymer matrices as supports have enhanced speed, sensitivity, and versatility in diagnostics of desired analytes. Conducting polymers are the conjugated polymers that can be synthesized by chemical methods as well as electrochemical methods, provide easy modulation of various properties (e.g., film thickness, conductivity, functionalization, use of various supporting electrolytes, ability to serve as an electrochemical transducer itself). Additional merits include entrapping of enzyme molecules during electropolymerization in one step and also uniform covering of the surface of substrate electrodes of any shape or size by polymer film [71,72].

Table 4 provides a comparison of analytical properties of conducting polymer-based AChE biosensors.

Sol–gel-based AChE biosensors

Sol–gel matrices have been known for their rigidness, chemical inertness, thermal and photochemical stability, negligible swelling in aqueous solution, tunable porosity, and optical transparency. Besides, they have been widely used in the fabrication of chemical optoelectronic sensors because most of the biological materials tend to retain their activity owing to the attractive low-temperature process of immobilization for various biomolecules (e.g., enzymes, antibodies).

Sol–gel supports used for immobilization of enzyme: Sol–gel crystals derived from tetramethyl orthosilicate (TMOS) [73], sol–gel film on a glass cap [74], silica sol–gel [37], TMOS sol–gel film [40,75], chromoionophore (ETH5294) doped sol–gel film [76],

Table 3
AChE biosensors based on nonconducting polymers for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/ Inhibitors	Incubation time (min)	Storage stability (days)	Reference
MWCNTs/PAN membrane/Pt electrode	Amperometric	Affinity bonds using concanavalin A	5.0×10^{-9}	3.6×10^{-8}–3.6×10^{-5}	Paraoxon	20	120	[59]
PAN/AuNPs/Pt electrode	Amperometric	Covalent	0.026×10^{-5}	3.6×10^{-7}–3.6×10^{-4}	Paraoxon	20	30	[60]
MSF–PVA/GCE	Amperometric	Entrapment	0.2×10^{-3}	0.2×10^{-3}–44.8×10^{-3}	Monocrotophos	10	30	[63]
PVA–SbQ polymer/SPE	Amperometric	Entrapment	1.91×10^{-2} 1.24×10^{-3}	NR	Paraoxon and chlorpyrifos-ethyl oxon	10	NR	[38]
PVA–SbQ membrane/ Pt electrode	Amperometric	Entrapment	7.2×10^{-5}, 0.18, and 0.049	NR	Paraoxon, maneb, and thifensulfuron methyl	30	30	[52]
PAMAM–gold/CNTs/ GCE	Amperometric	Electrostatic interaction	4.0×10^{-3}	4.8×10^{-3}–9.0×10^{-2}	Carbofuran	9	21	[64]

Note. NR, not reported.

Table 4
AChE biosensors based on conducting polymers for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/ Analyte/Inhibitors	Incubation time(min)	Storage stability (days)	Reference
Polyacrylamide membrane/pH electrode	Amperometric	Crosslinking	3.62×10^{3}	NR	Dichlorvos	30	50	[53]
PEI-coated GCE	Potentiometric Flow injection measurement	Covalent	1.0	NR	Dichlorvos	10	NR	[65]
PEI/SPE	Amperometric	Noncovalent	1.0×10^{-4}	NR	Dichlorvos	2 days	NR	[41]
Mercaptobenzothiazole/PANI/ Au electrode	Amperometric	Adsorption	0.48×10^{-3} 0.61×10^{-3}	NR	DiazinonFenthion	20	NR	[66]
PANI/CNT wrapped with ssDNA/Au electrode	Electrochemical	Covalent	1.0×10^{-6}	1.0×10^{-5} and 1.0	Methyl parathion and chlorpyrifos	15	5	[67]
AuNPs–PPy nanowires composite film modified GCE	Electrochemical	Entrapment	7.5×10^{-3}	0.018–0.45 and 1.89–17.0	Methyl parathion	12	30	[68]
PPy and PANI copolymer doped with MWCNTs/GCE	Amperometric	Adsorption	3.02×10^{-3}	0.030–1.51 and 3.027–75.67	Malathion	15	30	[69]
ZnS and poly(indole-5-carboxylic acid)/Au electrode	Amperometric	Covalent	0.1×10^{-3} and 1.5×10^{-3}	0.1–50×10^{-3} and 1.5–40×10^{-3}	Malathion and chlorpyrifos	10	60	[70]

Note. NR, not reported.

Al_2O_3 sol–gel matrix [77], sol–gel matrix on 7,7,8,8-tetracyanoquinodimethane (TCNQ) [78], AuNPs–SiSG [79], alumina sol–gel [80], bromothymol blue doped sol–gel film [81], zinc oxide sol–gel [82], and silica sol–gel film [83].

Merits: Apart from the entrapment of sensing agents without enzyme leaching and longer biomolecule stability, the advantages of sol–gel glasses include short response time and easy fabrication of multianalyte detection electrodes.

Demerits: Diffusional limitations, reproducibility of results, sensitivity, nonconductive nature, and unknown catalyst–matrix interactions and kinetics.

Table 5 provides a comparison of analytical properties of sol–gel-based AChE biosensors.

SPE-based AChE biosensors

The development of screen-printed biosensors involves the immobilization of the biological receptor in an active form onto the electrode surface. Such different immobilization procedures have been evaluated for the fabrication of SPEs in various configurations. Because the analytical performance of the electrode is strongly affected by this process, intensive efforts must be made to develop effective immobilization methods for improved operational and storage stability, response time, linear range, sensitivity, and preserved enzyme affinity for the substrates and/or inhibitors.

Supports used for immobilization of enzyme: TMOS sol–gel film/ SPE [40], Al_2O_3 sol–gel matrix SPE [77], sol–gel matrix on TCNQ

Table 5
AChE biosensors based on sol–gel for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/ Inhibitors	Incubation time (min)	Storage stability (days)	Reference
Sol–gel crystals derived from TMOS	Optical	Encapsulation	0.94 42.19	3.17–31.48 14.89–998.40	NaledMecarbam	5	30	[73]
Sol–gel film on a glass cap	Fiber-optic	Encapsulation	0.098	0.098–0.55	Paraoxon	30	NR	[74]
Silica sol–gel/SPE	Amperometric	Encapsulation	0.024, 0.015, and 0.012	0.01–0.001	Paraoxon, dichlorvos, and chlorpyrifos-ethyl oxon	20	6	[37]
TEOS sol–gel/GCE	Amperometric	Encapsulation	0.008	0.008–0.81	Oxydemeton methyl	20	21	[75]
TMOS sol–gel film/SPE	Amperometric	Encapsulation	1.0×10^{-3}	1.0 and 3.0×10^{3}	Dichlorvos	15	NR	[40]
Chromoionophore (ETH5294) doped sol–gel film	Optical fiber	Encapsulation	2.26	2.26–31.67	Dichlorvos	15	NR	[76]
AuNPs–SiSG/GCE	Electrochemical	Hydrogen bonds	0.44	NR	Monocrotophos	10	30	[79]
Alumina sol–gel/sonogel–carbon electrode	Amperometric	Encapsulation	2.5×10^{-4}	0.5	Chlorpyriphos-ethyl oxon	10	50	[80]
Bromothymol blue doped sol–gel film	Optical fiber	Encapsulation	0.11	0.14–5.70	Chlorpyrifos	8	60	[81]
Zinc oxide sol–gel/SPE	Amperometric	Electrostatic interactions	0.127	0.127–5.010	Paraoxon	10	90	[82]
Silicasol–gel film/carbon paste electrode	Amperometric	Encapsulation	3.0×10^{-4} and 0.47	3.7×10^{-4}–1.8×10^{-3} and 0.27–4.09	Methyl parathion and acephate	20 and 4	30	[83]

Note. NR, not reported.

modified SPE [78], SPE (TCNQ mediator [7,7,8,8-tetracyanoquinonedimethane] in the graphite electrode) [36], Prussian blue (PB) modified SPE [1], cobalt(II) phthalocyanine (CoPC)/SPE [84], *o*-phenylenediamine onto carbon/CoPC SPE [85], graphite–epoxy composite/SPE [86], SPE [87], PVA–SbQ polymer/SPE [38], and SWCNT–CoPC/SPE [88].

Merits: SPEs offer a number of advantages over conventional electrodes such as they are suitable for working with microvolumes and are to easier prepare and modify. They are reusable and inexpensive and have excellent specificity and selectivity. SPEs have important advantages such as the elimination of memory effects in the analysis at trace levels, and they appear to be particularly attractive for in situ determinations. The construction of SPEs involves the printing of different inks on planar ceramic or plastic supports. The great flexibility of SPEs resides in their large number of possible modifications. In fact, the composition of the inks used in the printing process can be modified by adding substances of a very different nature such as metals, enzymes, polymers, and complexing agents.

Table 6 provides a comparison of analytical properties of amperometric SPE-based AChE biosensors.

Quantum dot-based AChE biosensors

Quantum dots (QDs) are semiconductor particles that have all three dimensions confined to nanometer-length scales [89]. Recently, they have been widely used in biosensing and bioconjugates due to their size-dependent properties and dimensional similarities with biological macromolecules [90,91].

Supports used for immobilization of enzyme: CdTe QDs/AuNPs/chitosan (CHIT)/GCE [79], CdTe QDs/Au electrode [92], and poly(allylamine hydrochloride)/CdTe QDs/glass [93].

Merits: QDs are highly luminescent photostable fluorophores that have recently been used for sensing applications. QDs have much higher photoluminescence quantum efficiency than their bulk counterparts. Hybrid systems containing QDs coupled with various biomolecules have stimulated research in biotechnology and nanotechnology. A subtle change of the surface property of QDs can result in a dramatic change in their optical properties. In principle, this novel feature of QDs can be extended for detecting specific analytes if appropriate conditions are established.

Table 7 provides a comparison of analytical properties of QD-based AChE biosensors.

Nanoparticle-based AChE biosensors

Nanoparticle (NP)-based AChE biosensors have many advantages both in terms of stability and in terms of promoting the catalytic reduction of redox species. In addition, the electrode is notable for its ability to inhibit the oxidation of interfering species. NPs have attracted much interest owing to their unique properties such as high mechanical strength, oxygen ion conductivity, biocompatibility, and retention of biological activities [45]. NPs have electroactive surface of electrode, resulting in enhanced electron transport between electrolyte medium and the electrode.

Supports used for immobilization of enzyme: AuNPs–$CaCO_3$ bioconjugate/Au electrode [94], Fe_3O_4NP/MWCNTs/Au electrode [95], Fe_3O_4NP/MWCNTs/indium tin oxide (ITO) electrode [96], AuNPs/PB/GCE [97], MWCNTs–gold nanocomposites/GCE [98], ZrO_2/CHIT composite film/GCE [99], gold–platinum bimetallic NPs/GCE [45], AuNPs/GCE [100], AuNPs–MWCNTs/GCE [101], PB and CHIT/GCE [43], TiO_2-decorated grapheme/GCE [102], graphite, nanoplatelet–CHIT composite/GCE [103], calcium carbonate–CHIT composite film/GCE [104], CdS-decorated graphene nanocomposite [105], CHIT–GNPs/Au electrode [106], MWCNTs–CHIT composite/GCE [42], AuNPs/gold electrode [107], MWCNTs/AuNPs–CHIT/GCE [18], PbO_2/TiO_2/Ti [108], and PB–CHIT/GCE [109].

Table 6
AChE biosensors based on screen-printed electrodes for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/Inhibitors	Incubation time (min)	Storage stability (days)	Reference
Al_2O_3 sol–gel matrix SPE	Amperometric	Adsorption	0.01	0.1–80	Dichlorvos	15	5	[77]
Sol–gel matrix on TCNQ modified SPE	Amperometric	Entrapment	1×10^{-2} 8×10^{-4} and 2×10^{-2}	NR	Carbaryl, carbofuran, and pirimicard	20	45	[78]
SPE (TCNQ mediator in the graphite electrode)	Amperometric	Adsorption	3.0×10^{-6}	5×10^{-2}–0.2	Chlorpyrifos-ethyl oxon	10	50	[36]
PB modified SPE	Amperometric	NR	0.126, 0.124, 7.2×10^{-3}, and 1.6×10^{-3}	0.063–0.315, 0.124–0.497, 7.2×10^{-3}–18.1×10^{-3}, and 1.6×10^{-3}–6.5×10^{-3}	Aldicarb, carbaryl, paraoxon, and chlorpyrifos-methyl oxon	30	21	[1]
CoPC/SPCEs	Amperometric	Crosslinking	4.9×10^{-4}	10^{-5}–1.0	Carbofuran	15	NR	[84]
o-Phenylenediamine onto carbon/CoPC SPE	Amperometric	Entrapment	1×10^{-11}, 1×10^{-10}, and 1×10^{-10}	1.0×10^{-11}–1.0×10^{-2}	Dichlorvos, parathion, and azinphos	10	92	[85]
Graphite–epoxy composite/SPE	Amperometric	Crosslinking	1.0×10^{-4} and 1.0×10^{-5}	NR	Paraoxon and carbofuran	15	5	[86]
SPE	Amperometric	Crosslinking	0.18	0.18–54.00	Paraoxon	10	NR	[87]
Single-walled CNTs–CoPC/SPE	Amperometric	Covalent	0.01 and 6.3×10^{-3}	0.018–0.181 and 6.36×10^{-3}–0.159	Paraoxon and malaoxon	15	3	[88]

Note. NR, not reported.

Table 7
AChE biosensors based on quantum dots for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/ technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/ Analyte/ Inhibitors	Incubation time (min)	Storage stability (days)	Reference
CdTe QDs/AuNPs/CHIT/GCE	Amperometric	Covalent	1.34	4.4×10^{-3}–4.48 and 8.96–67.20	Monocrotophos	8	30	[92]
CdTe QDs/Au electrode	Amperometric	Covalent	2.98×10^{-3}	4.96×10^{-3}–2.48	Carbaryl	10	30	[106]
Poly(allylamine hydrochloride)/CdTe QDs/glass	Optical	Electrostatic interaction	1.05×10^{-5} and 4.47×10^{-6}	1.0×10^{-6}–1.0 and 1.0–0.1	Paraoxon Parathion	15	35	[93]

Merits: Metal NPs (e.g., Au, Ag, Pt, Cu) have large surface areas and excellent conductivity and catalytic properties, making them suitable as carriers to immobilize enzymes and active materials to fabricate efficient modified electrodes. Among these metal NPs, AuNPs have been widely used to immobilize enzymes resulting from their good biocompatibility and preservability of enzymatic activities.

Table 8 provides a comparison of analytical properties of NP-based AChE biosensors.

Applications of AChE biosensors

The majority of AChE biosensors are designed to detect AChE inhibitors (OP pesticides and heavy metals) in environmental and food matrices. AChE biosensors also show promise in public safety. The basic design of these devices, the electrode material, and the immobilization chemistry used to attach the enzyme determine their performance and operational characteristics. Most AChE sensors designed for practical applications use immobilized enzyme. The primary application of AChE biosensors is for the detection of pesticides. Pesticides bind to the esterase active site of the enzyme and inhibit the catalytic activity. AChE is inhibited by both OP and carbamate pesticides, but the mechanisms of inhibition are different. In the latter case (carbamates), the inhibition is slightly reversible, whereas in the former case most OP pesticides induce an irreversible inhibition. In the case of irreversible inhibition, the AChE can be reactivated with oxime-type reactivation agents such as pyridine-2-aldoxime methachloride [110]. This mechanism is applied for the reactivation of AChE in a biosensor design, which makes possible repetitive use of the same biosensor, after successive inhibition measurements [34].

Summary and conclusion

AChE biosensors are strong candidates for screening pesticide residues and are becoming more and more relevant in environmental and food analysis. Compared with traditional chromatography

Table 8
AChE biosensors based on nanoparticles for pesticide detection.

Electrode material/ Immobilization matrix	Type of transducer/technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/Inhibitors	Incubation time (min)	Storage stability (days)	Reference
AuNPs–$CaCO_3$ bioconjugate/ Au electrode	Amperometric	Adsorption	0.1×10^{-3}	0.1×10^{-3}–100×10^{-3} and 0.1×10^{-3}–70×10^{-3}	Malathion and chlorpyrifos	10	90	[70]
Fe_3O_4NP/MWCNTs/Au electrode	Amperometric	Covalent	0.1×10^{-3}	$0.1–40 \times 10^{-3}$, $0.1–50 \times 10^{-3}$, $1–50 \times 10^{-3}$, and $10–100 \times 10^{-3}$	Malathion, chlorpyrifos, monocrotophos, and endosulfan	10	60	[95]
Fe_3O_4NP/MWCNTs/ITO electrode	Amperometric	Covalent	0.1×10^{-3}	$0.1–70 \times 10^{-3}$, $0.1–50 \times 10^{-3}$, $0.1–70 \times 10^{-3}$, and $0.1–100 \times 10^{-3}$	Malathion, chlorpyrifos, monocrotophos, and endosulfan	10	90	[96]
AuNPs/PB/GCE	Amperometric	Adsorption	3.5×10^{-9}	4.48×10^{-3}–4.48×10^{-2}	Monocrotophos	10	30	[97]
MWCNTs–Au nanocomposites/GCE	Amperometric	Hydrophilic surfacefor biomolecule adhesion	1.81×10^{-3}	3.0×10^{-3}–3.027	Malathion	8	30	[98]
ZrO_2/CHIT composite film/ GCE	Amperometric	Absorption	1.3, 5.0×10^{-3}, and 1.7	6.6–440, 0.01–0.59, and 8.6–520	Phoxim, Malathion, and imethoate	15	30	[99]
Gold–platinumbimetallic NPs/GCE	Amperometric	Crosslinking with glutaraldehyde	50×10^{-3}, 40×10^{-3}, and 40	$50–200 \times 10^{-3}$, l.40–50×10^{-3}, and 40–60	Paraoxon ethyl, sarin, and aldicarb	25	NR	[45]
AuNPs/GCE	Amperometric	Adsorption	7.0×10^{-3}	28×10^{-3}–170×10^{-3}	Methamidophos	10	7 days	[100]
AuNPs–MWCNTs/GCE		Adsorption	1.0×10^{-3}	0.1×10^{-3}–7.0×10^{-3}		30	NR	[101]
PB and CHIT/GCE	Amperometric	Glutaraldehyde crosslinking	0.113×10^{-4}, 0.703×10^{-4}, 0.194×10^{-4}, and 0.33×10^{-4}	0.45×10^{-4}–0.045, 0.234×10^{-3}–0.046, 0.116×10^{-3}–0.0194, and 0.167×10^{-3}–0.0335	Dichlorvos, omethoate, trichlorfon, and phoxim	10	NR	[43]
TiO_2-decorated grapheme/ GCE	Amperometric	Adsorption	1.4×10^{-3}	4.9–74.5 and $74.5–9.9 \times 10^{3}$	Carbaryl	3	20	[102]
Graphitenanoplatelet–CHIT composite/GCE	Voltammetric	Covalent	1.58×10^{-4}	1×10^{-4}–1.0	Chloropyrifos	10	10	[103]
Calciumcarbonate–CHIT composite film/GCE	Electrochemical	Entrapment	3.7×10^{-3}	0.018–0.759 and 2.84–14.24	Methyl parathion	10	NR	[104]
CdS-decorated graphene nanocomposite	Amperometric	Adsorption	3.4×10^{-3}	9.9×10^{-3}–9.93	Carbaryl	2	20	[105]
CHIT–GNPs/Au electrode	Amperometric	Chemisorption/esorption	0.1×10^{-3}	0.3×10^{-3}–60.5×10^{-3}	Malathion	15	NR	[106]
MWCNTs–CHITcomposite/ GCE	Amperometric	Covalent	NR	NR	Carbaryl, malathion, dimethoate, and monocrotophos	8	30	[42]

Table 8 (*continued*)

Electrode material/ Immobilization matrix	Type of transducer/technique	Immobilization method	Detection limit (μM)	Linearity (μM)	Application/Analyte/Inhibitors	Incubation time (min)	Storage stability (days)	Reference
AuNPs/Au electrode	Amperometric	Adsorption	33×10^{-3}	10×10^{-3}–135×10^{-3}	Carbofuran	20	7	[107]
MWCNTs/AuNPs–CHIT/GCE	Fourier transform continuous cyclic voltammetry	Adsorption	0.01	0.1–10	Monocrotophos	NR	50	[18]
PbO_2/TiO_2/Ti	Amperometric/Flow injectionanalysis system	Adsorption	0.1×10^{-3}	0.01–20	Trichlorfon	10	5	[108]
PB–CHIT/GCE	Amperometric	Covalent	3.0×10^{-3}	0.01–0.4 and 1.0–5.0	Carbaryl	10	30	[109]

Note. NR, not reported.

and other methods, the strengths of AChE biosensors are that they are very selective, sensitive, and disposable and also work with complete automation and provide rapid results.

Attempts have been made to summarize the salient features of various acetylcholine biosensors reported so far. Most of the reports have focused on the use of AChE for detection of specifically organophosphate and carbamate pesticides. AChE sensors have the potential to be a billion-dollar market, and the technology needs improvement in biological stability, signal transduction, precision, and cost-effectiveness. The role of matrices for biosensing and the characteristics of various biosensors, in terms of response time, detection limit, and linear range, have been delineated. It is suggested that microfabrication technology and nanoengineering do indeed have an enormous and profitable impact on the pesticide biosensor market.

Future perspectives

The development of cheap and disposable array biosensors for the simultaneous detection of clinically important metabolites and rapid screening of pesticides is still needed. The use of biomolecules to grow NPs has great promise in the future of biosensing and design of bioelectronic systems. We believe that the use of biocatalytic nanostructure growth, using dip pen nanolithography as a patterning method and biomolecules as templates for nanostructure synthesis, holds great promise in future nanotechnologies.

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