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A Review of Recent Developments in Acetylcholinesterase Biosensors

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Abstract: Over the past decades, acetylcholinesterase (AChE) biosensors have emerged as a highly simple, rapid, and ultra-sensitive technique for pesticide analysis in environmental monitoring, food safety, and quality control. In this paper, we will highlight the research efforts over the last ten years in fabricating AChE biosensors, the recent trends and future prospects for the development of better AChE biosensing systems. Research on various immobilization methods, different strategies for biosensor construction, the performance and application of constructed AChE biosensors are extensively introduced in this review. *Copyright © 2013 IFSA.*

Keywords: Acetylcholinesterase biosensors, Inhibition, Electrochemical, Pesticide residues, Immobilization.

1. Introduction

Pesticides play an important role in the high productivity achieved in agriculture through insects control [1-3]. However, the presence of pesticide residues in food, water, and soil has become a major Issue in environmental chemistry. Long-term accumulations in environment, organophosphate and carbamate insecticides (Fig. 1) will present a serious risk to human health due to its high toxicity to acetylcholinesterase, a key enzyme for the function of the central nervous system in humans [4-8]. To protect human health from possible hazards, it is necessary to develop sensitive, fast, and reliable methods for determination of pesticide residues in food, water, and soil. Traditional analytical methods, such as liquid chromatographic [9, 10], gas chromatography [11] and high performance liquid chromatography (HPLC) [12] are most commonly employed to trace pesticide residues and also are part of regulations in monitoring the environmental pollutants. However, these methods require extensive sample preparation, specialized analytical equipment, and technical expertise, which are all unsuitable for rapid, immediate and large-scale sample analysis. Thus, the development of efficient, sensitive and simple analytical method for the determination of pesticide residues in food has become increasingly important.

AChE biosensors meet these requirements. Compared with various methods, AChE biosensors emerged as an ultra sensitive and selective technique for pesticide residues monitoring for environmental, agricultural and food applications (Table 1). AChE biosensors based on the inhibition action of pesticides on AChE have shown satisfactory results, in which the enzymatic activity is used as an indicator of quantitative measurement of insecticides [35-37]. The strategy of AChE biosensor for the pesticide detection is shown in Fig. 2 [38].

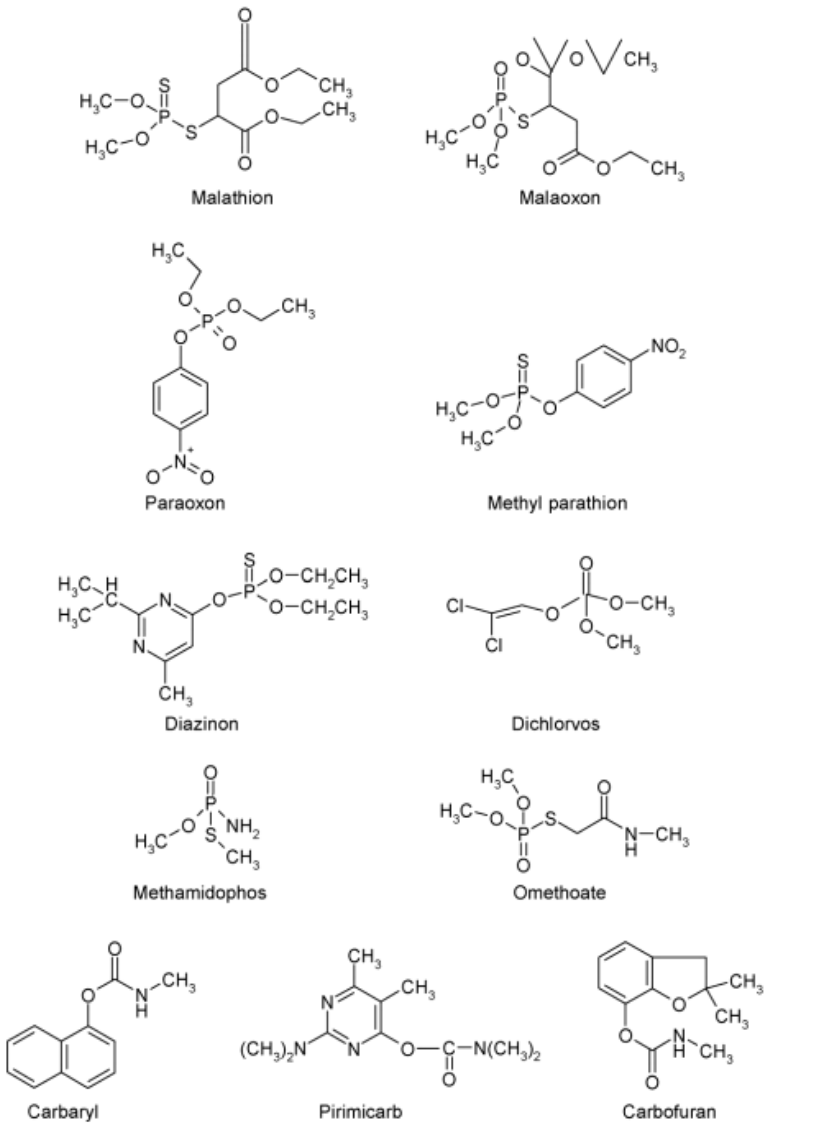

Fig. 1. Structures of the main pesticides used as targets in AChE biosensors.

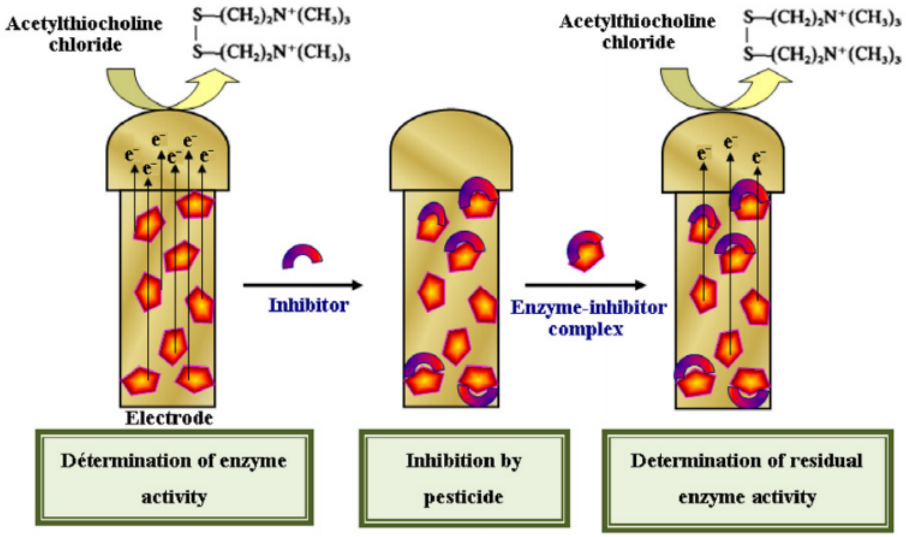

Fig. 2. The strategy of AChE biosensor for the pesticide detection.

Table 1. Design of AChEs for biosensor applications.

Electrode material	Technique	Immobilization method	Detection limit (μM)	Inhibitors	Incubation time (min)	Reference
Glass/sol-gel indicator/ polyvinylidene fluoride membrane	Fiber-optic	Crosslinking with glutaraldehyde	0.53 and 0.023	Carbaryl Dichlorvos	10	[13]
Cellophanemembrane/ AuE	Amperometric	Crosslinking	1.45	Paraoxon	15	[14]
MWCNTs/PAN membrane/ Pt electrode	Amperometric	Affinity bonds using concanavalin A	5.0×10^{-9}	Paraoxon	20	[15]
PAN/AuNPs/Pt Electrode	Amperometric	Covalent	0.026×10^{-5}	Paraoxon	20	[16]
MSF-PVA/GCE	Amperometric	Entrapment	0.2×10^{-3}	Monocrotophos	10	[17]
PEI/SPE	Amperometric	Noncovalent	1.0×10^{-4}	Dichlorvos	2 days	[18]
PPy and PANI copolymer doped with MWCNTs/GCE	Amperometric	Adsorption	3.02×10^{-3}	Malathion	15	[19]
TEOS sol-gel/GCE	Amperometric	Encapsulation	0.008	Oxydemeton methyl	20	[20]
TMOS sol-gel film/SPE	Amperometric	Encapsulation	1.0×10^{-3}	Dichlorvos	15	[21]
AuNPs-SiSG/GCE	Electrochemical	Hydrogen bonds	0.44	Monocrotophos	10	[7]
Bromothymol blue doped Sol-gel film	Optical fiber	Encapsulation	0.11	Chlorpyrifos	8	[22]
CoPC/SPCEs	Amperometric	Crosslinking	4.9×10^{-4}	Carbofuran	15	[23]
SPE	Amperometric	Crosslinking	0.18	Paraoxon	10	[24]
CdTe QDs/AuNPs/CHIT/GCE	Amperometric	Covalent	1.34	Monocrotophos	8	[25]
CdTe QDs/Au electrode	Amperometric	Covalent	2.98×10^{-3}	Carbaryl	10	[26]
AuNPs/PB/GCE	Amperometric	Adsorption	3.5×10^{-9}	Monocrotophos	10	[27]
AuNPs/GCE	Amperometric	Adsorption	7.0×10^{-3}	Methamidophos	10	[28]
TiO_2-decorated grapheme/ GCE	Amperometric	Adsorption	1.4×10^{-3}	Carbaryl	3	[29]
Graphitenanoplatelet-CHIT composite/GCE	Voltammetric	Covalent	1.58×10^{-4}	Chloropyrifos	10	[30]
Calciumcarbonate-CHIT composite film/GCE	Electrochemical	Entrapment	3.7×10^{-3}	Methyl parathion	10	[31]
CHIT-GNPs/Au electrode	Amperometric	Chemisorption/ esorption	0.1×10^{-3}	Malathion	15	[26]
CdS-decorated graphene nanocomposite	Amperometric	Adsorption	3.4×10^{-3}	Carbaryl	2	[32]
AuNPs/Au electrode	Amperometric	Adsorption	33×10^{-3}	Carbofuran	20	[33]
PB-CHIT/GCE	Amperometric	Covalent	3.0×10^{-3}	Carbaryl	10	[34]

Acetylcholinesterases (AChE) are a class of enzymes that hydrolyze the neurotransmitter acetylcholine in the nervous system. The reaction that is catalysed by AChE is: acetylcholine + H_2O → choline + acetate; 2thiocholine → dithio-bis-choline + $2H^+$ + $2e^-$ [39, 40]. AChE has a very high catalytic activity and each molecule of AChE can degrade approximately 25,000 molecules of acetylcholine per second [38]. The catalytic reaction occurs when the triad's anionic binding site attracts the positively charged quaternary ammonium group of acetylcholine (structure is seen in Fig. 3). However, in the presence of an inhibitor such as organophosphate and carbamate insecticides, the catalytic reaction would be blocked. The AChE is a biorecognition element, which is sensitive to be inhibition by organophosphates and carbamate pesticides. And AChE has been widely used as an effective recognition element for the configuration of biosensors for pesticides detection [41]. AChE are most commonly available to extract from the Drosophila melanogaster and the Electric Eel and employed to construct biosensor. AChE have different substrate specificity and susceptibility to inhibitors, which depend on the extraction source [42]. Studies of AChE biosensors for pesticides detection have yielded several practical outcomes [43-56].

In this paper, we specifically provide an overview of the research carried out over the last ten years relative to various AChE immobilization methods, different strategies for biosensor construction, the performance and application of constructed AChE biosensors. Research on the trends and challenges associated with AChE biosensor for practical applications, and the future prospects for development of better AChE biosensing systems are extensively introduced in this review.

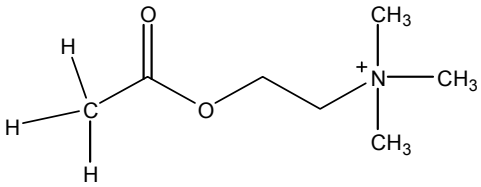

Fig. 3. Structure of acetylcholine.

2. AChE Immobilization

The most essential step in the development of an enzyme biosensor is the effective immobilization of the enzyme onto the surface of the modified electrode. It influence the performance of the biosensor such as stability, sensitivity, selectivity, response time and reproducibility. The performance of the biosensor declines if immobilization causes enzyme denaturation or conformational changes, especially on its active site. To maintain the inherent nature of enzyme, it is necessary to choose effective AChE immobilization methods. There are a variety of strategies by which enzymes can be immobilized: adsorption, covalence, entrapment, cross-linking and affinity [38, 42, 57-59] (Fig. 4, Fig. 5). The main advantages and drawbacks of each immobilization method was presented in Table 2.

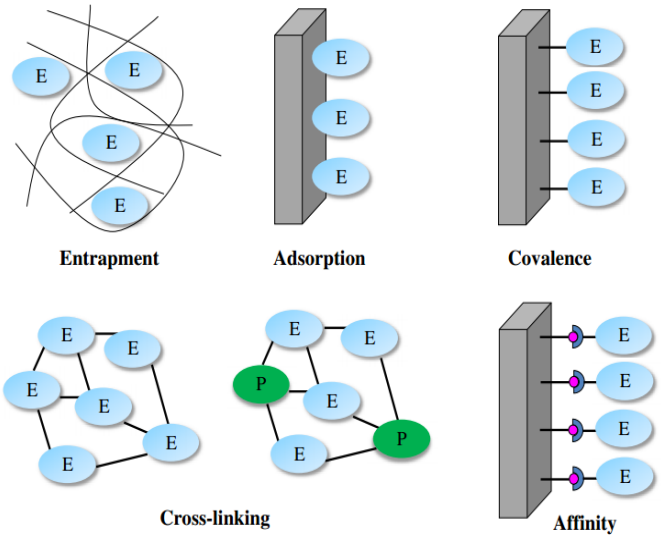

Fig. 4. Schematic representation of the main different methods of enzyme immobilization. E: enzyme, P: inert protein.

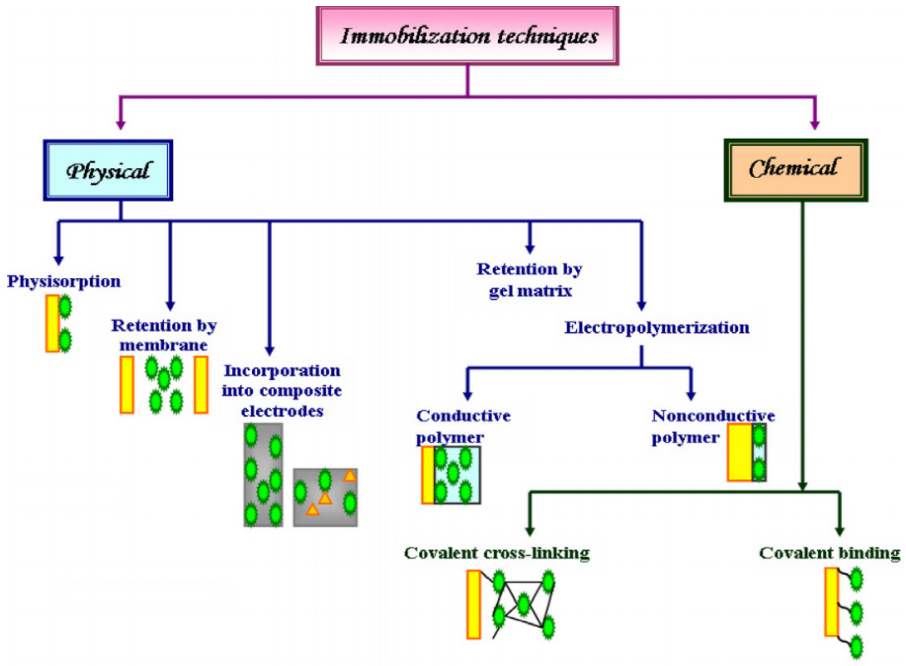

Fig. 5. Immobilization techniques used for the development of AChE biosensors.

Table 2. Advantages and drawbacks of the five basic immobilization methods.

	Binding nature	Advantages	Drawbacks
Adsorption	Weak bonds	Simple and cheap, no damage to enzyme, no chemical change	Enzyme Leakage, nonspecific binding, sensitive to environment
Covalent coupling	Chemical binding between functional groups of the enzyme and those on the support	Absence of diffusion barriers, short response time, no enzyme leakage	High amount of enzyme, possible denaturation, coupling with toxic product
Entrapment	Incorporation of the enzyme within a gel, a polymer or membrane	One-step procedure, suitable for a large variety of enzymes, simple	Enzyme Leakage, diffusion barriers, many biocompatible polymers available
Cross-linking	Bond between enzyme/cross-linker/inert molecule	Simple	High enzyme activity loss
Affinity	Af finity bonds between a functional group on a support and af finity tag on a protein sequence	Reusable surface, low amount of enzyme, controlled and orientated immobilization	Need of specific groups in the bioreceptor molecule

2.1. Physical Entrapment

AChE can be immobilized in three-dimensional matrices such as electropolymerized films or sol-gel matrices. Table 3 presents performances of some biosensors based on AChE-entrapping matrices. Electropolymerization, as a one-step method, consists in applying an appropriate potential or current to the transducer soaked in an aqueous solution containing both enzyme and monomer molecules. Finally, enzyme molecules are physically incorporated within the growing polymer network. Conducting polymers such as polyaniline, polypyrrole and polythiophene are often used as electropolymerized films for biomolecule immobilization (Fig. 6). The sol-gel process is a synthetic inorganic procedure. Typically, the procedure involves hydrolysis of alkoxide precursors in the acidic or alkaline conditions, followed by condensation of the hydroxylated units, which leads to the formation of a silica gel network around the enzyme (Fig. 7) [59].

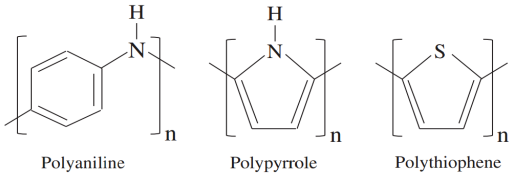

Fig. 6. Polyaniline, polypyrrole and polythiophene structures.

This immobilization can be done by an easy one-step fabrication of the electrode, in which enzyme, mediators and additives can be simultaneously deposited in the same sensing layer. The activity of the enzyme is preserved during the immobilization process because of the absence of chemical bond. However, the method has some limitations such as leaching of biocomponent and possible diffusion. Increased operational and storage stability is often used to characterize AChE biosensors based on physically entrapped enzymes.

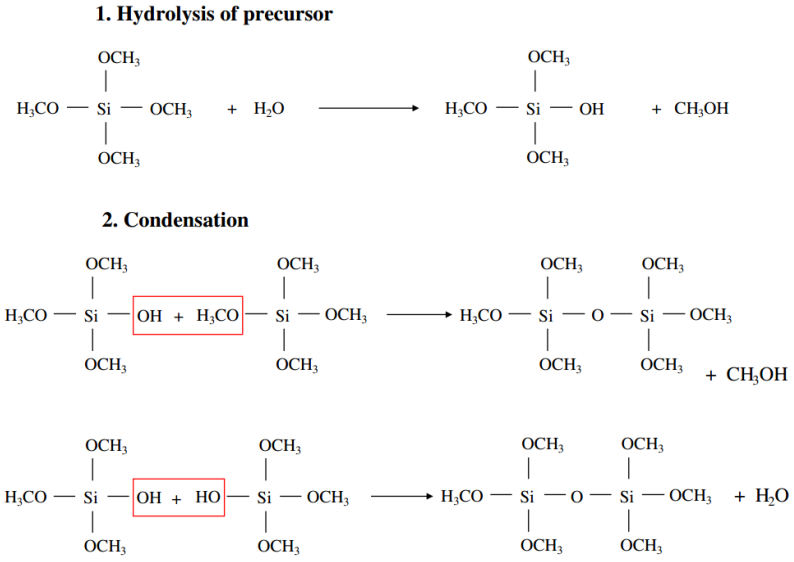

Fig. 7. Sol-gel process composed by two steps: hydrolysis of TMOS (1), condensation of silanol groups (2).

Table 3. Biosensors based on AChEs entrapped within a polymer.

Analyte	Detection mode	Detection limit (M)	Linearity range (M)	Reference
Chlorpyrifos ethyl oxon	Amperometry	1.24×10^{-9}	-	[70]
Paraoxon	Amperometry	1.91×10^{-8}	-	[70]
Dichlorvos	Amperometry	7×10^{-11}	-	[71]
Dichlorvos	Amperometry	9.6×10^{-11}	2×10^{-10}-1×10^{-8}	[72]
Omethoate	Amperometry	1×10^{-7}	Up to 3×10^{-6}	[73]
Carbaryl	Amperometry	1×10^{-8}	-	[74]

2.2. Physical Adsorption

Physical adsorption consists of deposition of AChE onto the surface of modified electrode and attachment through weak bonds such as Van der Waals forces and electrostatic interactions between the AChE and the modified electrode. This method has widely been thought to the easiest and least denaturing strategy. It does not involve any functionalization of the electrode materials or covalent links.

This technique has been successfully used to immobilize AChE onto the surface of screen-printed graphite disposable electrodes [60-62]. Bonnet's group had used a series of suitable washing buffers to wash the electrodes, which removed the superficial and the excess of adsorbed enzyme. In this system, the electrodes were stable for eight continuous assays. After a 50-day storage under vacuum, the electrodes conserved the same activity.

2.3. Covalent Coupling

AChE can be immobilized onto the surfaces of a transducer for biosensors application via a stable covalent bond between functional groups of AChE and the transducer. The procedure involves initial activation of the surface of the transducer with a bifunctional cross-linker such as glutaraldehyde, carbodiimide or succinimide, followed by enzyme coupling to the activated support through amino, carboxyl or hydroxyl groups, then the removal of excess and unbound biomolecules. The method provides increased stability of the enzyme, but requires high amounts of bioreagent and is poor in reproducible.

There are many researches about utilizing this approach to immobilize AChE with glutaraldehyde [63-65] or carbodiimide [66] Nhydroxysuccinimide (NHS) can be associated with carbodiimide to improve the immobilization efficiency of AChE (Fig. 8) [59].

2.4. Cross-Linking

Immobilization of AChE by cross-linking with bifunctional agents, such as glyoxal, glutaraldehyde or hexamethylenediamine is widely used to develop biosensors. In this procedure, enzymes are cross-linked with each other or in the presence of a functionally inert protein. The superiority of this method is its simplicity and the strong chemical binding achieved between biomolecules. However, the activity of AChE may lose due to the distortion of its conformation and the chemical alterations of its active site.

Amperometric biosensor was developed using the cross-linking method to immobilize AChE and choline oxidase (ChO) on an electrosynthesized bilayer membrane modified Pt electrode [67]. Detection limits (at S/N = 3) in flow injection analysis were 100 nM for both acethylcholine and choline at the ChO-AChE sensor. This device is developed for the simultaneous detection of acethylcholine and choline. It is potentially useful for the analysis of real matrices, such as brain homogenates and CSF, without the use of a chromatographic step typically required to separate interferents electroactive at the detection potential of hydrogen peroxide.

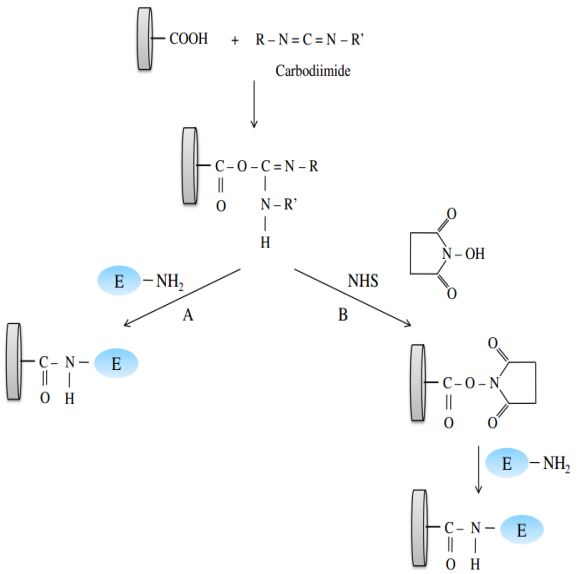

Fig. 8. Enzyme immobilization on carboxylated surface by carbodiimide coupling (A) without or (B) with NHS.

2.5. Oriented Immobilization of AChE via an Affinity Tag

This method provides a basis for controlled and oriented immobilization of the enzyme on different supports, which can avoid enzyme deactivation and/or active site blocking, providing a way to solve the problem of minimizing the loss of enzyme activity during the immobilization procedure. Progresses have been achieved in developing biosensors based on oriented and site-specific immobilization of enzymes. The key procedure is to create (bio) affinity bonds between an activated support, namely functional groups (lectins, avidin or metal chelates) of an activated electrode surface, and a specific group (an affinity tag, with carbohydrate residue, biotin or histidine) of the protein sequence (not affect the activity or the folding of the protein). In general, AChE contain limited affinity tags in its protein sequence, which meet requirements for affinity binding. Therefore, it is necessary to attach affinity tags to the protein sequence by genetic engineering methods such as site-directed mutagenesis, protein fusion technology and post-transcriptional modification [42], usually to an amino or carboxyl terminal group that is far enough from the active site [68, 69].

At present, three kinds of affinity methods have been described to immobilize enzymes. Biotin-(strept) avidin, a method use the strong affinity existing between biotin and (strept) avidin (dissociation constant of 10−15 M) to immobilize enzymes. Metal cation-chelator, a strategy use the strong affinity link between a metal cation and a chelator such as nitrilotriacetic acid (NTA), imidodiacetic acid (IDA) or tag poly(histidine) to develop enzymatic biosensors. Lectin-carbohydrate was between a sugar moiety naturally present in some enzymes such as AChE and concanavalin A (Con A) deposited onto the surface a modified electrode. The procedure of oriented immobilization of AChE via an affinity tag for pesticides detection was shown in Fig. 9. Biosensors based on AChE immobilized by affinity was also presented in Table 4.

3. Classification of AChE Biosensors

3.1. Membrane-Based AChE Biosensors

AChE biosensors based on membranes such as polyacrylamide membrane [78], nylon and cellulose nitrate membrane [79], poly (2-hydroxyethyl methacrylate) membrane [80], cellophane membrane [14], hybrid mesoporous silica membrane [81] and poly-(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) (PAN) membrane [15, 16], offer a portable, cheap, and rapid method for the determination of pesticides.

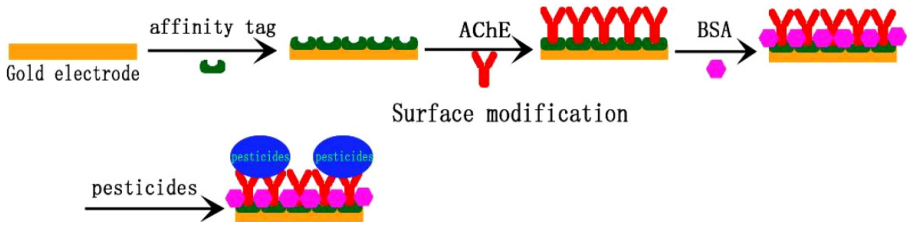

Fig. 9. Schematic representation of oriented immobilization AChE.

Table 4. Biosensors based on AChEs immobilized by affinity.

Analyte	Immobilization strategy	Detection method	Detection limit (M)	Linearity range (M)	Reference
Acetylthiocholine	NTA derivative	Amperometry	-	1×10^{-6}-3×10^{-4}	[68]
Paraoxon	NTA	Amperometry	2×10^{-9}	-	[75]
Chlorpyrifos	Con A	Amperometry	1×10^{-8}	-	[76]
Acetylthiocholine	Con A	Amperometry	-	1×10^{-5}-1.1×10^{-4}	[76]
Acetylthiocholine	Con A	Amperometry	-	1×10^{-5}-1×10^{-4}	[77]
Chlorpyrifos methyl-oxon	Con A	Amperometry	5×10^{-8}	-	[75]

The membrane-based AChE biosensors have solved several problems such as loss of enzyme, maintenance of enzyme activity, reduction in time of the enzymatic response and increased shelf life of the biosensor because of the high degree of flexibility, mechanical durability and wider pH range for use of the membrances immobilized. Immobilization methods of AChE onto modified polymer membranes was shown in Fig. 10 [82].

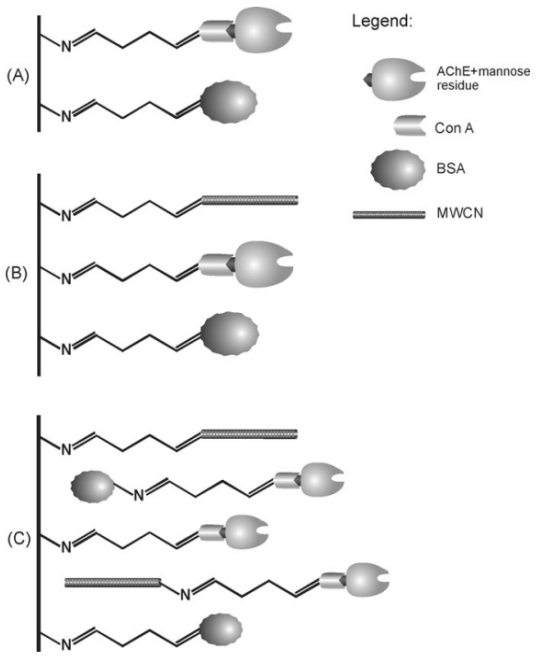

Fig. 10. Immobilization methods of enzyme onto modified polymer membranes: (A) immobilization via glutaraldehyde (method 1), (B) immobilization via glutaralde-hyde and Con A (method 2), (C) immobilization via glutaraldehyde, mixture of MWCN + BSA, glutaraldehyde and Con A (method 3).

3.2. Polymeric Matrix-Based AChE Biosensors

Polymeric matrix-based AChE biosensors are divided into two main categories based on the conductivity of polymeric matrix. AChE biosensors based on nonconducting polymer matrices have been reported by many research groups [15-17, 70, 83, 84]. Such biosensors are easy to prepare, and can prolong the storage of enzyme because of the propertity of nonconducting polymer matrices providing a favorable microenvironment for the enzyme molecules. Many researches (polyethylenimine (PEI)-coated GCE [85] Polyacrylamide membrane/pH electrode [78] AuNP-polypyrrole (PPy) nanowire composite film modified GCE [86] mercaptobenzothiazole/polyaniline (PANI)/Au electrode [87] PPy and PANI copolymer doped with MWCNTs/GCE [19] have proven that conducting polymer matrices have merits such as enhanced speed, sensitivity, and versatility in diagnostics of desired analytes. What's more, enzyme molecules can be immobilized onto the electrode by electropolymerization in one step. The procedure of conducting polymeric matrix-based biosensors was shown in Fig. 11 [88].

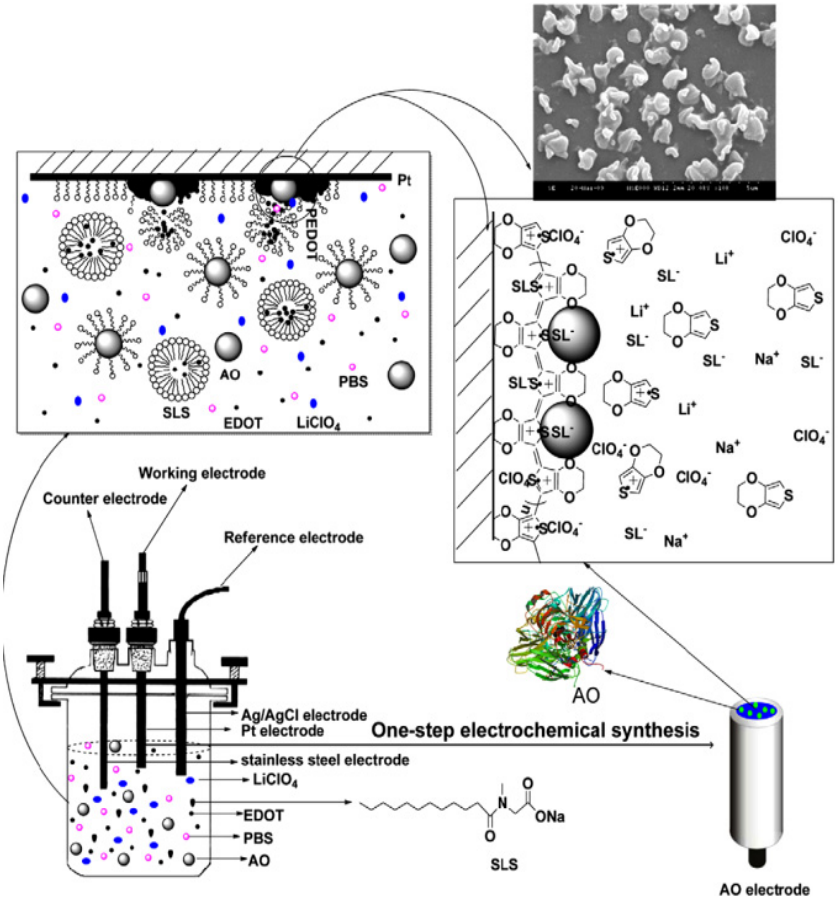

Fig. 11. Structures of EDOT, PEDOT, SLS, AO, and reaction mechanism during the electrosynthesis of the PEDOT composite film on the bare Pt disk electrode interface and SLS interaction with AO and EDOT in PBS. electrosynthesis of the PEDOT composite film and reaction catalyzed by AO.

3.3. Sol-Gel-Based AChE Biosensors

Sol-gel matrices have been known for its porous optically transparent matrix and demonstrated functional activity of encapsulated enzymes. However, conventional sol-gel procedures are unsuitable for the encapsulation of enzymes because of high acidic condition and/or high concentration of alcohol, which can denature the nature of enzyme. Recent years enormous progress [7, 22, 35, 74, 89-92] has obtained in the sol-gel-based enzyme biosensors by modifying conventional methods and developing mild conditions so that proteins maintain their native structure and characteristic activities. To design an effective biosensor, a good optical quality and stable sol-gel-based glassy matrix is required.

3.4. SPE-Based AChE Biosensors

Screen-printed electrodes (SPEs) offer a number of advantages over conventional electrodes such as design flexibility, process automation, good reproducibility, a wide choice of materials and the elimination of memory effects in the analysis at trace levels. The construction of SPEs consists of layer-by-layer depositions of ink upon planar ceramic or plastic supports, through the use of a screen or mesh, defining the geometry of the sensor. Enzymes can be immobilized in an active form onto the surface of the working electrode, which can be of different nature such as gold or carbon, through adsorption, entrapment, cross-linking or covalent attachment [93, 94]. In order to improve operational and storage stability, response time, linear range, sensitivity, and preserve enzyme affinity for the substrates and/or inhibitors, intensive efforts is necessary to made. The composition of the inks used in the printing process can be modified by adding substances of a very different nature such as metals, polymers, enzymes, and complexing agents, which increases the flexibility of SPEs. SPE-based biosensors are shown as practical devices for the rapid, easy-to-use and low cost determination of many substances [23, 24, 35, 74, 95-97]. The typical configurations used in designing screen-printed biosensors was shown in Fig. 12 [98].

3.5. Nanomaterials-Based AChE Biosensors

Nanomaterials and nanofabrication technologies are increasingly being used to design novel biosensors. The sensitivity and specificity of biomolecule detection and the capability of detecting or manipulating atoms and molecules are being improved by using nanomaterials for their construction. Nanomaterials-based biosensors represent the integration of material science, molecular engineering, chemistry and biotechnology. Because of their submicron dimensions, nanosensors have allowed simple and rapid analyses in vivo. Nanomaterials, including nanotubes, nanofibers, nanorods and nanoparticles, are being gradually applied to biosensors because of their unique properties such as high mechanical strength, large surface areas, biocompatibility, oxygen ion conductivity and retention of biological activities [99]. Various kinds of nanomaterials, such as gold nanoparticles [100], carbon nanotubes (CNTs) [101], graphene [102] magnetic nanoparticles [103] and quantum dots [104, 105], have been widely used for biosensors.

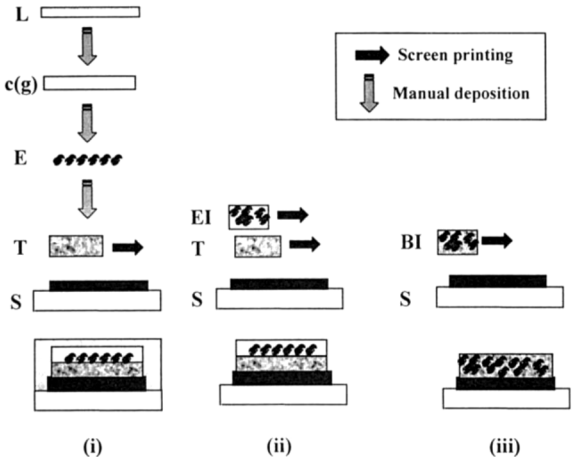

Fig. 12. Typical configurations used in designing screen-printed biosensors. (i) Multiple layers by manual deposition, (ii) screen printing of enzymatic inks or pastes using two or more steps, (iii) one-step configurations based on biocomposites ink or pastes. In all of these configurations, layers are applied on a substrate with conducting tracks previously formed by planar technologies. BI: biocomposite ink printed; c: layer of material used for immobilization (glutaraldehyde, etc) deposited by manual deposition; E: enzyme layer (manual deposition); EI: enzymatic ink or paste deposited by screen printing; L: outer protective layer (CA, Nafion, etc.) by manual deposition; S: substrate (PVC, ceramic, etc.) with conducting tracks formed previously; T: conductive paste deposited by screen printing. The mediators, stabilisers and additives, depending on the device, can be included in any ink or paste.

Recently, Sun et al., developed a novel AChE biosensor for direct determination of pesticide residues by immobilizing AChE on chitosan/prussian blue/multiwall carbon nanotubes/hollow gold nanospheres (Chit-PB-MWNTs-HGNs) nanocomposite film by one-step electrodeposition [106]. The incorporation of MWNTs and HGNs into Chit-PB hybrid film promoted electron transfer reaction, enhanced the electrochemical response and improved the microarchitecture of the electrode surface. Schematic illustration of the stepwise nanomaterials-based AChE biosensors fabrication process and TEM image of HGNs are shown in Fig. 13 and Fig. 14.

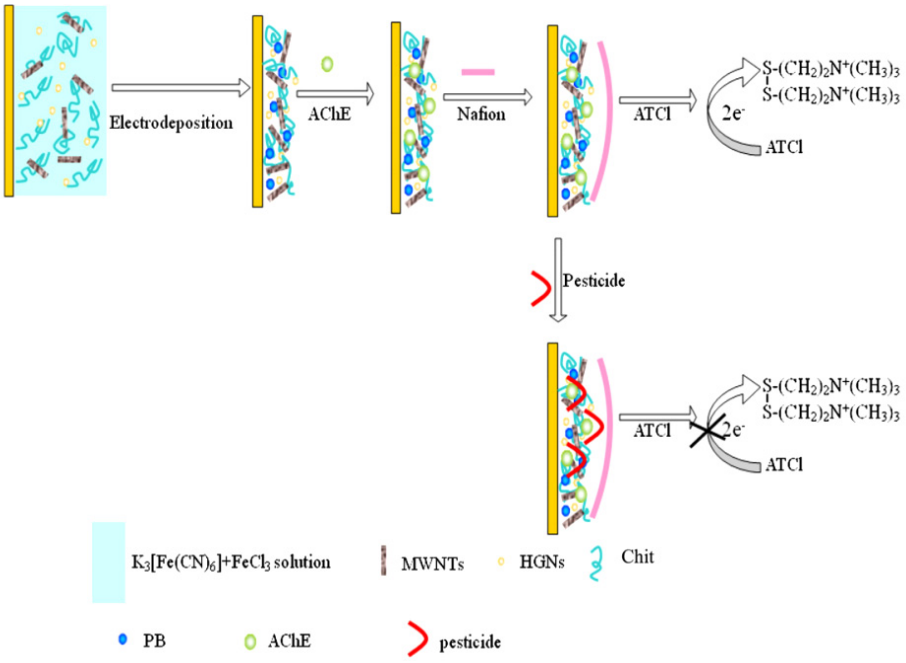

Fig. 13. Schematic illustration of the stepwise AChE biosensor fabrication process and immobilized AChE inhibition in pesticide solution.

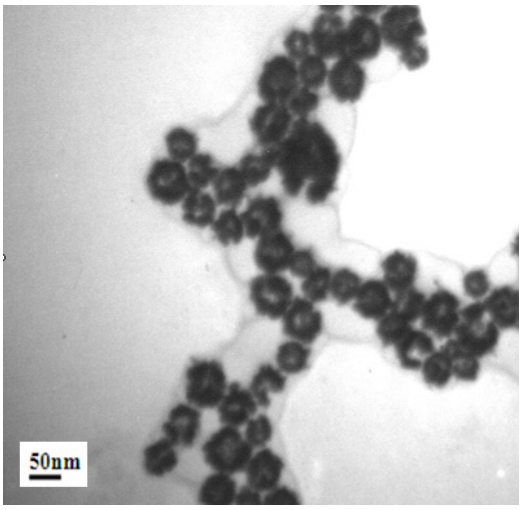

Fig. 14. TEM image of HGNs.

4. Practical Applications, Challenges and Prospects

The majority of AChE biosensors are designed to detect pesticides and heavy metals in environmental and food matrices. AChE biosensors show promise in public safety and military. For the applications of pesticides detection, amperometric biosensors are more sensitive than potentiometric biosensors [42]. There are many factors such as the electrode configuration, the immobilization technique used to attach the enzyme and incubation time of the pesticides with the enzyme affect the detection limit. Paraoxon, dichlorvos, diazinon, aldicarb and carbofuran are the most widely studied pesticides. Some pesticides, which have no or little inhibitory effect on AChE in their pure form, need to be oxidized to oxidation state. It is important to mention that traditional chromatographic methods are not able to distinguish between the two forms, although they differ by a single atom in their molecule.

However, most AChE biosensors reported in literature have been tested on standard solutions and not on real samples. The practical application of immobilized AChEs has a significant limitation due to the inhibition decrease the activity of AChEs. So enzyme reloading or reactivation is needed to reuse the biosensor, which will complicate the operation, especially in situ measurements. A solution to this problem is to employ single use disposable electrodes such as biosensors based on screen-printed electrode. The other limitation of existing AChE biosensors is related to their inability to identify particular analytes, so the selectivity for measuring AChE inhibitors is very poor [107, 108]. In general, all organophosphosphorus and carbamate pesticides, and heavy metals inhibit the activity of AChE. Hence, AChE biosensors are mainly promising for measuring the total toxicity of the sample, which will reflect the sum of all AChE inhibitors present in the sample. Another limitation of present AChE biosensors for practical applications is the transfer from pristine research laboratory conditions to commercial applications.

In the future, AChE biosensors could serve as an alternative to traditional chromatographic methods for multiple toxic analytes. The development of automated and continuous systems for measuring pesticides in flow conditions is an important area of research in AChE biosensors [77, 109, 110]. The

development of cheap and disposable array biosensors for the detection of AChE inhibitors is still needed. In recent years, the applications of nanomaterials in biosensors will provide novel opportunities for biosensor technologies.

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