Int. J. Electrochem. Sci., 10 **(2015)** 713 - 724

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Short review

Carbon Nanostructures for Development of Acetylcholinesterase Electrochemical Biosensors for Determination of Pesticides

Ning Xia[*], *Yanping Gao*

College of Chemistry and Chemical Engineering, Anyang Normal University, Anyang, Henan 455000, People's Republic of China
[*]E-mail: xianing82414@163.com

Received: 14 October 2014 / *Accepted:* 22 November 2014 / *Published:* 2 December 2014

The large amount of pesticide residues in the environment is a threat to global health by inhibition of acetylcholinesterase (AChE). Electrochemical biosensors based on inhibition of AChE are attractive for the detection of pesticides. To improve the performance characteristics of the biosensors for pesticides detection, carbon nanostructures such as carbon nanotubes (CNTs) and graphene allow for large quantities of enzyme to be immobilized, provide a favorable microenvironment to maintain the enzyme activity, and facilitate the oxidation of the enzymatic products. Recently, research on carbon nanostructures-based electrochemical biosensors began very recently and rapidly gained attention due to the outstanding properties of carbon nanostructures. The aim of this review is to provide insight into the carbon nanostructures-based electrochemical biosensors by inhibition of AChE and to show their benefits for determination of pesticides.

Keywords: Acetylcholinesterase; Pesticides; Electrochemistry; Biosensors; Carbon nanotubes; Graphene

1. INTRODUCTION

Pesticides are widely used in agriculture due to their high insecticidal activity. Unfortunately, frequent use of pesticides in agricultural lands worldwide has resulted in their appearance as residuals in crops, livestock, and poultry products. Their toxicity is based on the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), a hydrolase that hydrolyzes the neurotransmitter acetylcholine. Accumulation of acetylcholine in the synaptic cleft can lead to severe muscular paralysis and even death. Because exposure to even small amounts of pesticides may be fatal, public concern about the development of detection devices for effectively monitoring pesticides has grown steadily [1]. To date, the techniques of gas chromatography, liquid chromatography and thin film chromatography coupled with different types of detectors are the most commonly used methods for pesticides detection. These methods are rather time consuming and need very expensive equipment, highly trained personnel and complicated sample pretreatment(s) although they provide fruitful results [2, 3]. In contrast, electrochemical biosensors with high sensitivity, long term stability and low cost detection of specific biological binding events have extensively reduced sampling and testing times in pesticides determination. Recently, monitoring of AChE activity with electrochemical techniques has been widely employed as a mean for quantitative measurement of pesticides.

The combination of enzymatic reactions with the electrochemical method of monitoring electroactive enzymatic products allowed to develop enzyme based electrochemical biosensors for sensitive and rapid determination of pesticides [4-6]. These biosensors have used either AChE alone or combined with choline oxidase (ChO). The AChE inhibition in the single and bienzyme systems is monitored by measuring the oxidation current of the product of the enzyme reaction. In the single enzyme system (equation 1), AChE hydrolyzes acetylthiocholine (a synthesized analogues of acetylcholine) to produce the electrochemically active thiocholine, which undergoes electrochemical oxidation process at electrodes. In the bienzyme system (equations 2 and 3), AChE catalyzes the hydrolysis of acetylcholine into acetate and choline. The choline is subsequently converted by choline oxidase (ChO), producing hydrogen peroxide in the presence of oxygen. The produced hydrogen peroxide can be detected amperometrically with different electrochemical transducers. The approach based on the single enzyme system is largely preferred due to the inherent simplicity and robustness of a single enzyme system. However, the direct electrochemistry of thiocholine at solid electrode often needs large anodic potential (~ 0.65 V vs. Ag/AgCl) that cause high background current and interference from other electroactive compounds. Thus, various types of electrodes and electrode materials have been prepared in literature to facilitate the oxidation of thiocholine.

$$\textbf{acetylthiocholine} + H_2O \xrightarrow{\textbf{AChE}} \textbf{thiocholine + acetate acid} \quad \textbf{(1)}$$

$$\textbf{acetylcholine} + H_2O \xrightarrow{\textbf{AChE}} \textbf{choline + acetate} \quad \textbf{(2)}$$

$$\textbf{choline} + O_2 \xrightarrow{\textbf{ChO}} \textbf{betaine aldehyde} + H_2O_2 \quad \textbf{(3)}$$

Electrochemical biosensors based on the inhibition of AChE are attractive for the detection of pesticides. The degree of inhibition is therefore correlated to the reduction in thiocholine oxidative current relative to the current measured in the absence of an inhibitor. In a typical configuration, the enzyme is deposited onto the surface of the working electrode and the activity of the enzyme is measured by adding the substrate to the solution. The most important step in the development of an AChE biosensor is the stable 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 term of sensitivity, stability, response time and reproducibility. In this context, appropriate selection of the electrode material and immobilization chemistry is essential for a reliable AChE biosensor. At first, entrapment in silica sol–gel matrices such as tetraethylorthosilicate (TEOS) or polymers (e. g. nafion, chitosan) provides a unique means to prepare a three-dimensional network suited for the encapsulation of AChE [7-17]. To overcome the problem of thiocholine oxidation at the sol-gels or polymers modified electrodes, mediators such as cobalt (II) phthalocyanine (CoPC) [8, 18], tetracyanoquinodimethane (TCNQ) [7, 9, 19], poly(3,4-ethylenedioxythiophene) (PEDOT) [20], tetrathiafulvalene–tetracyanoquinodimethane (TTF–TCNQ) [21] and Prussian blue (PB) [14-16] have been doped with the sol-gel or polymers in these works. The performances of these modified electrodes were shown in Table 1. Also, some alternative non-silica sol-gels with increased biocompatibility have been used for the immobilization of AChE. For example, Stanciu's group reported the design of AChE biosensors based on ZnO for pesticides detection [22]. Marty's group prepared a biosensor for chlorpyriphos-ethyl-oxon detection by entrapping AChE in Al_2O_3 sol–gel [23].

Table 1. Sol-gels and polymers for immobilization of AChE and detection of pesticides.

Electrodes	**Mediators**	**Pesticides**	**Detection limits**	**Refs.**
AChE/TMOS/graphite	TCNQ	carbaryl, carbofuran, pirimicard	10 nM, 0.8 nM, 20 nM	[9]
AChE/TEOS/SPE	CoPC	malathion, methidathion, dimethoate	1 nM, 10 nM, 5 μM	[8]
AChE/PEDOT:PSS/SPE	PEDOT	chlorpyrifos-oxon	4 nM	[20]
AChE/TEOS/carbon paste	—	methyl parathion, monocrotophos,	0.08 ppb, 87 ppb	[7]
AChE/TEOS/carbon paste	—	malathion, acephate	0.058 ppm, 0.044 ppm	[12]
AChE/IL gel/carbon paste	TTF–TCNQ	serine, neostigmine	26 pM, 0.3 nM	[21]
AChE/PB-chitosan/GC	PB	aldicarb, paraoxon, methyl parathion	30 ppb, 10 ppb, 5 ppb,	[14]
AChE/PB–chitosan/GC	PB	carbaryl	3 nM	[15]
AChE/PB–chitosan/GC	PB	dichlorvos, omethoate, trichlorfon, phoxim	2.5 ng/L, 15 ng/L, 5 ng/L, 10 ng/L	[16]
AChE/ZnO sol–gel/SPE	—	paraoxon	0.035ppm	[22]
AChE/Al_2O_3/carbon paste	—	chlorpyriphos-oxon	0.25 nM	[23]
AChE-ChO/Pt-CAs/BDD	—	methamidophos, monocrotophos	0.05 ppt, 0.6 ppt	[24]
AChE-PVA-AWP/SPE	CoPC	chlorpyriphos-oxon, ethyl paraoxon, malaoxon	5 pM, 5 nM, 0.5 nM	[18]
AChE/PAn/SPE	CoPC	dichlorvos, parathion, azinphos	0.01 fM, 0.1 fM, 0.1 fM,	[27]
AChE-ChO/PPy	—	paraoxon, carbofuran	1.1 ppb, 0.12 ppb	[25]

Recently, carbon aerogels (CAs) have attracted tremendous attention because of their many excellent properties such as high electrical conductivity, great mesopore volume and high accessible surface area; Liu and Wei reported the preparation of AChE/Pt-CAs modified boron-doped diamond (BDD) electrode and demonstrated its application in the detection of methamidophos and monocrotophos [24]. Moreover, instead of the mediators-doped electrodes, the conducting polymers such as polypyrrole (PPy) and polyaniline (PAn) have been widely used as the enzyme-hosting matrix in electrochemical biosensors because of their advantages of exhibiting relatively high conductivity, easy preparation, and excellent environmental stability [25, 26]. AChE can be entrapped inside the polymers matrix or attached to the surface of polymers film. The biosensors with conducting polymers as the supports obviate the need of extra mediators for promoting the oxidation of enzymatic products.

To improve the performance characteristics of the AChE-based biosensors for pesticides detection, electrode materials used should allow for large quantities of enzyme to be immobilized, provide a favorable microenvironment to maintain the enzyme activity, and facilitate the oxidation of the enzymatic products. Recently, carbon nanomaterials have been employed in the construction of electrochemical biosensors to improve the analytical performances. In this work, we summarized the carbon-based nanostructures for fabrication of AChE-based electrochemical biosensors and demonstrated their applications in determination of pesticides, including carbon nanotubes (CNTs), graphene and their hybrid nanocomposites.

2. CNTs AND HYBRID CNTS NANOCOMPOSITES

2.1 CNTs

Carbon nanotubes (CNTs) consisting of several concentric tubes of graphite inside one other have proven to be a powerful class of catalyst supports due to their large surface area, unique structural and electromechanical properties, good biocompatibility, ease of preparation and surface renewability [28]. The first AChE-based electrochemical biosensor with CNTs as the electrode supports was reported by Lin *et al.* in 2004 [29]. In the work, AChE and CHO were covalently attached to multiwall CNTs (MWCNTs)-modified screen-printed electrode. The biosensor exhibits a high sensitivity, large linear range, and low detection limits for pesticides detection due to the large surface area and high catalytic activity of MWCNTs to the oxidation of the produced H_2O_2. Lately, Joshi et al. reported a disposable biosensor based on AChE-functionalized MWCNTs modified thick film strip electrode for insecticides detection [30]. The large surface area and electro-catalytic activity of MWCNTs lower the overpotential for thiocholine oxidation to 200 mV (vs. Ag/AgCl). Furthermore, immobilization of enzyme to an electrode surface without its bioactivity being sacrificed is a crucial step for the design of the biosensors. The commonly used methods for immobilizing enzyme on electrodes include physical adsorption, covalent bonding or cross-linking, assembling techniques, physical entrapment and so on. Subsequently, different methods for immobilization of AChE on CNTs or CTNs-modified electrodes have been reported, including physical adsorption, covalent bonding or cross-linking, assembling techniques and entrapment in sol-gels or polymers (Table 2) [4, 31-35]. Typically, Lin’s group reported a method for AChE immobilization based on self-assembling AChE on the MWCNTs surface with cationic poly(diallyldimethylammonium chloride) (PDDA) as the linker [4]. The formation of layer-by-layer (LBL) nanostructures on the MWCNTs surface provides a favorable microenvironment to maintain the bioactivity of AChE and to prevent enzyme leaking. Du et al. proposed a simple method for covalently immobilizing of AChE to a composite of chitosan and MWNTs with glutaraldehyde as the cross-linker [31]. Furthermore, they reported the controllable adsorption of the MWCNTs onto the hexyl mercaptan ($C_6H_{13}SH$) self-assembled monolayers (SAMs)-covered gold electrode through hydrophobic interaction between MWCNT and the hydrophobic SAMs and onto the cysteamine (Cys)-covered nanoporous gold (NPG) electrode with the self-assembly technique [32]. AChE was then linked to the adsorbed MWCNTs for amperometric sensing of pesticides by inhibition of AChE.

As mentioned above, the application of mediators significantly decreases the working potential as well as improves reproducibility and sensitivity of thiocholine detection. Several groups have demonstrated the eletrocatalytic properties of mediator–CNT complexes for the detection of the produced thiocholine [36]. For example, Chen et al. suggested that MWNTs facilitated the electrochemical polymerization of prussian blue and enhanced the enzymatic activity of AChE [37]. Moraes et al. suggested that the MWCNT/CoPc-modified GC electrode showed excellent catalytic property of phthalocyanines without losing any of the electronic absorption property of MWCNT [38]. Rotariu et al. investigated the sensing performances of MWCNTs–TCNQ/sol-gel modified electrode [39].

The conducting polymers can provide suitable environment for immobilization of biomolecules and act as mediators. In the recent years, nanostructures formed by conducting polymers combined with CNTs have received great deal of attention. The resulting nanostructures not only embody the merits of the different components but also exhibited enhancement of electrical ability as well as mechanical properties compared with pure conducting polymer or CNTs. Du et al. reported the electropolymerization of PPy and PAn on CNTs modified electrode for immobilization of AChE [40]. Also, Cesarino et al. demonstrated the preparation of MWCNT/PAn film by electropolymerization in a solution containing a certain ratio of MWCNT/PAn on glassy carbon electrode. Poly(allylamine hydrochloride) (PAH) is a weak cationic polyelectrolyte with many ionizable amine groups in its backbone and has been widely used in sensor and biosensor fabrication. Çevik et al. reported the preparation of a novel biosensor based on AChE/MWCNT/PAH modified carbon paste (CP) electrode for detection of monocrotophos [41].

All of the above results were obtained with MWCNT that offer extended adsorption of proteins and auxiliary reagents. Being smaller and more sensitive to structural defects than multi-walled CNTs, SWCNTs form less regular layers onto the electrodes with a higher deviation of the signal measured. Several groups have also demonstrated the use of single-walled carbon nanotubes (SWCNTs) in fabrication of AChE-based biosensors. For example, Sharma et al. demonstrated the covalent immobilization of AChE onto the SWCNTs-modified ferrule graphite electrode (FGE) by cross-linking with glutaraldehyde [42]. Ivanov et al. presented the design of the AChE biosensor based on a screen-printed electrode covered with SWCNTs together with the CoPc mediator [36]. Viswanathan et al. described the fabrication of a novel electrochemical biosensor by integrating the special features of ssDNA–SWCNT, PAn and AChE for pesticides determination [43].

2.2 Hybrid CNTs nanocomposites

Metallic/metallic oxide nanoparticles have been used to aid the immobilization of enzymes in the preparation of electrochemical biosensors. Various electrodes modified with the metallic/metallic oxide nanoparticles and CNTs have also led to the development of electrochemical sensors for pesticides detection. Herein, we summarized the applications and the performances of the nanocomposites of CNTs and metallic/metallic oxide modified electrodes.

Because of the unique chemical and physical properties of AuNPs, AuNPs have been widely used in design of electrochemical sensors [44, 45]. They are considered to be the excellent candidates for replacing potentially harmful mediators in the construction of biosensors and act as the hopping bridge of electrons generated from the redox reaction [44, 46]. Several groups have demonstrated that the use of AuNPs enhanced the immobilization of AChE on an electrode surface and facilitated the electrochemical oxidation of the produced thiocholine. The AuNPs-based AChE biosensors allowed for the detection of pesticides with low detection limits (Table 2) [47-51]. In these works, AuNPs could be anchored on electrode surface by electrochemical reduction of $HAuCl_4$, embedding in hydrogels or by assembling on SAMs-covered gold electrodes. Moreover, Du's group also demonstrated that the immobilized AChE can induce the growth of colloidal AuNPs on the electrode surface via the reduction of $HAuCl_4$ by the produced thiocholine [47]. The resulting AuNPs as the mediators improved the electron transfer between $[Fe(CN)_6]^{3-/4-}$ and electrode. For these views, AuNPs-CNTs nanostructures have been successfully used to enhance the sensitivity of AChE biosensors [52, 53]. For example, the dendrimers polyamidoamine (PAMAM) can provide a suitable microenvironment to retain the AChE activity; Qu et al. reported the detection of pesticides using the self-assembled AChE/PAMAM-Au/CNTs multilayer electrode [53]. Furthermore, Hou et al. presented an amperometric biosensor for acetylcholine detection based on self-assembly of AuNPs and AChE on the MWCNTs/ChOx/sol–gel-modified Pt electrode surface [54].

Among a wide variety of metal oxide nanoparticles, Fe_3O_4 nanoparticles have been used increasingly in many electrochemical applications due to their unique magnetic and electrical properties. Nano-sized magnetic bioconjugated materials have been used in electrochemical biosensor devices due to many potentially unique properties such as large surface area, higher bioactivity, excellent conformation stability and better contact between biocatalyst and its substrate. The integration of CNTs and Fe_3O_4 nanoparticles is expected to provide a synergistic effect in construction of AChE biosenors. For example, Chauhan et al. developed a strategy of immobilizing covalently AChE onto Fe_3O_4/MWCNT modified gold electrode and addressed its application in construction of an amperometric biosensor for determination of pesticides [55, 56]. This sensor offered high sensitivity, high biocompatibility, high charge transfer rate and good stability.

Other metallic oxide nanoparticles such as zirconium dioxide (ZrO_2), zinc oxide (ZnO) and silicon dioxide (SiO_2) were also deposited on the CNTs-modified electrode surface for construction of AChE-based biosensors. For example, ZrO_2 is an ideal support for immobilization of biomolecules because of its low toxicity, excellent chemical inertness and biocompatibility; Pundir et al. reported the construction and application of a bienzymatic choline sensor by covalently immobilizing AChE and ChO onto nanocomposites of carboxylated MWCNTs and ZrO_2 nanoparticles [2]. ZnO is a wide-used semiconductor with a band gap of 3.37 eV and possesses attractive electronic and optical properties; ZnO nanoparticles were deposited on a negatively charged MWCNTs layer at the electrode surface, which led to a highly sensitive and extremely stable choline and acetylcholine sensor [57]. Moreover, copper oxide (CuO) is an important transition metal oxide with potential applications in sensors due to its enhanced catalytic and electrical properties. Cu or Cu-containing compounds have good affinity with thio-compounds. For this consideration, Huo et al. realized the direct detection of pesticides using CuO nanowires (NWs) and SWCNTs modified glassy carbon electrode [58]. These results *are* endowed with the promising future of developing AChE-based biosensors for pesticides detection using the hybrid carbon/metallic oxide nanocomposites.

Table 2. CNTs nanocomposites modified electrodes for pesticides detection.

Electrodes	Pesticides	Detection limits	Refs.
AChE-ChOx/MWCNT/SPE	methyl marathion	0.05 μM	[29]
AChE/MWCNT/SPE	paraoxon	0.5 nM	[30]
AChE/PDDA/MWCNT/GC	paraoxon	0.4 pM	[4]
AChE/chitosan-MWNTs/GC	triazophos	0.01μM	[31]
AChE/IL–MWCNT gel/CPE	chlorpyrifos	4 nM	[35]
AChE/MWNTs/SAMs-Au	carbaryl	0.6 ng/mL	[32]
AChE/MWNTs/Cys-NPG	malathion	0.5 ng/mL	[59]
AChE/PB/MWNT/GC	carbofuran	0.04 ppb	[37]
AChE/CoPc-MWCNT/GC	carbaryl	5.46 nM	[38]
AChE/CoPc-MWCNT/SPE	paraoxon, malaoxon	3 ppb, 2 ppb	[36]
AChE/sol gel-TCNQ-MWCNT/SPE	methyl paraoxon, chlorpyrifos	30 pM, 0.4 nM	[39]
Nafion/AChE/Chitosan–PB–MWNTs–HGNs/AuE	malathion, chlorpyrifos, monocrotophos, carbofuran	0.05 nM, 0.05 nM, 0.1 nM, 2.5 nM	[60]
AChE/PAn-PPy-MWCNTs/GC	malathion	1.0 ng/mL	[40]
AChE/PANI-MWCNT/GC	carbamate	4.6 nM	[61]
AChE/PANI-MWCNT/GC	carbaryl, methomyl	1.4 μM, 0.95 μM	[62]
AChE/PAH-MWCNT/CPE	monocrotophos	0.88 pg/mL	[41]
AChE/SWCNT–PANI/Au	methyl parathion, chlorpyrifos	10 nM, 0.01 μM	[43]
AChE/AuNPs/Au	carbofuran	33 nM	[47]
AChE/AuNPs/sol-gel/chitosan/GCE	monocrotophos	0.6 ng/ml	[48]
AChE/AuNPs/PDMS/chitosan/Au	Malathion, monocrotophos	0.001 μg/mL	[49]
AChE/AuNPs–PPy/GC	methyl parathion	2 ng/mL	[51]
AChE/AuNPs–SF/Pt	methyl paraoxon, carbofuran, phoxim	0.02 nM, 0.1 nM, 2 nM	[50]
AChE/PAMAM-AuNPs–CNTs	carbofuran	4 nM	[53]
AChE-ChO/PtNPs–CNT/SPE	diazinon oxon	—	[63]
AChE/Fe_3O_4-MWCNT/Au	malathion, chlorpyrifos, monocrotophos, endosulfan	0.1 nM, 0.1 nM, 1 nM, 10 nM	[55]
AChE/Fe_3O_4-MWCNT/ITO	malathion, chlorpyrifos, monocrotophos, endosulfan	0.1 nM	[56]

3. GRAPHENE AND HYBRID GRAPHENE NANOCOMPOSITES

3.1 Graphene

The performance of the AChE electrochemical biosensors mainly depends on two key factors, the activity of the immobilized AChE and the electron transfer rate of the electrode. Therefore, scientist focused on the introduction of advanced functional materials for electrode interface construction and enzyme immobilization. Graphene, as a new two-dimensional carbon nanomaterial, has attracted increasing attention during recent years because of its physical and chemical properties and excellent electrocatalytic ability [64]. Thus, it shows excellent application potential in nanoelectronic devices and sensors for pesticides detection (Table 3). Because the reduced graphene oxide (GRO) could well maintain the specific bioactivity of biomolecules and exhibit catalytic ability to small molecules, Wu et al. demonstrating the application of GRO/nafion in AChE loading for the electrochemical sensing of pesticides [65]. Compared with graphene, porous reduced graphene oxide (pRGO) shows improved performance in electronic devices and sensors. pRGO should also be very useful as a new support because porous supports can not only provide high surface area but also facilitate the diffusion and mass transport of reactants [66]. Li et al. demonstrated the preparation of pRGO/chitosan modified electrode and addressed its application in detection of AChE inhibitors. Very recently, Zhang et al. reported the synthesis of N_{α},N_{α}-bis (carboxymethyl)-L-lysine hydrate (NTA-NH_2)-Ni^{2+} functionalized graphene oxide (FGO) with abundant affinity binding sites for histidine (His)-tagged AChE and demonstrated its application towards the fabrication of paraoxon biosensors [67].

3.2 Hybrid graphene nanocomposites

It has been reported that the integration of CNTs-based materials and metallic/metallic oxide nanoparticles usually shows synergistic effects in electrocatalytic applications, so there is a reason to expect the integration of graphene and metallic/metallic oxide nanoparticles has the similar effect on the electrooxidation of thiocholine. Liu et al. reported the immobilizing of AChE on reduced graphene oxide (rGO)/AuNPs modified electrode [68]. The enzyme retained high activity after being immobilized on the electrode by the boronic acid-diol interaction. The integration of rGO and AuNPs enhanced the signal of electro-oxidation of thiocholine. Zhang et al. proposed a stable AChE biosensor based on simple and efficient self-assembling AChE to graphene nanosheets (GNs)-AuNPs nanocomposite modified electrode for investigation of their inhibition, reactivation and aging processes of different pesticides [69]. In Du’s work, rGO was readily incorporated into the polypyrrole (PPy) by electrochemical scans [70]. AuNPs were subsequently electrodeposited onto the PPy–rGO surface. AChE was then encapsulated in a silica matrix and immobilized on the AuNPs–PPy–rGO nanocomposites by co-deposition with $(NH_4)_2SiF_6$. The biosensor led to the rapid and sensitive detection of paraoxon-ethyl from 1.0 nM to 5 mM with a detection limit of 0.5 nM. Besides AuNPs, Pt nanoparticles (PtNPs) have also attracted a great deal of attention due to their excellent conductivity. Carboxylic graphene (CGR) with a carboxyl functional group could be bonded with PtNPs. Yang et al.

demonstrated that the synthesized Pt NPs–CGR nanocomposites are easily dispersed in nafion solution and the electrode modified with PtNPs/CGR/NF film showed excellent conductivity, catalysis, and biocompatibility [71]. In the work, AChE was immobilized on PtNPs/CGR/Nafion-modified GCE with chitosan as cross-linker.

Nanocomposites of graphene and metal oxide nanoparticles such as ZnO, SnO_2, TiO_2, and ZrO_2 have also been synthesized and used for detection of pesticides. Typically, semiconductor nano-sized TiO_2 has been extensively used in a wide range of applications due to its non-toxicity, long term stability, low cost, multifunctions and good biocompatibility; Wang et al. described an amperometric biosensor for organophosphate compounds (OPs) based on immobilization of AChE on the TiO_2-decorated graphene (TiO_2-G) nanohybrid [72]. SnO_2 nanoparticle is an n-type semiconductor with a wide band gap of 3.6 eV at 300 K; Zhou et al. reported a sensitive amperometric acetylcholinesterase (AChE) biosensor with SnO_2/CGR/nafion modified glassy carbon electrode for the detection of methylparathion and carbofuran [73]. Moreover, Wang et al. demonstrated that ZnO/CGR nanocomposites could be dispersed in nafion solution and the resulting ZnO-CGR/nafion possessed excellent conductivity, catalysis and biocompatibility [74, 75]. AChE could be attached onto the ZnO-CGR/nafion modified electrode surface by the hydrophilic interaction.

Table 3. Graphene nanocomposites modified electrodes for pesticides detection.

Electrodes	**Pesticides**	**Detection limits**	**Refs.**
AChE/GRO/GC	dichlorvos	2.0 ng/mL	[65]
AChE/pRGO/GC	carbaryl	0.5 ng/mL	[66]
AChE/Ni-NTA–cGO/GC	paraoxon	0.65 nM	[67]
AChE/AuNPs–rGO/GC	chlorpyrifos, malathion, carbofuran, isoprocarb	0.1 ppb, 0.5 ppb, 0.05 ppb, 0.5 ppb	[68]
AChE/AuNPs–GO/GC	paraoxon	0.1 pM	[76]
AChE/GNs–AuNPs/SPE	paraoxon-ethyl, monocrotophos, methomyl	—	[69]
AChE/Au–PPy–rGO/GC	paraoxon-ethyl	0.5 nM	[70]
AChE/PtNPs–CGR/GC	methyl parathion, carbofuran	0.05 pM, 0.5 pM	[71]
AChE/TiO_2-G/GC	carbaryl	0.3 ng/mL	[72]
AChE/SnO_2–CGR/GC	methyl parathion, carbofuran	0.05 pM, 0.5 pM	[73]
AChE/ZnO–CGR/GC	chlorpyrifos, carbofuran	0.05 pM, 0.52 pM	[74]

4. CONCLUSION

Nanomaterials are attractive because of their unique electrical, chemical and physical properties. Carbon nanomaterials such as CNTs and graphene have been especially targeted for developing AChE-based biosensors. By utilizing CNTs and graphene, the biosensors have shown great promise for the detection of pesticides, primarily because the two nanostructured electrode materials are used as signal transducers to mediate current flow. Most of the existing limitations of the AChE-based electrochemical biosensors could be directly related to the selectivity in multicomposite mixtures and complex matrices and the inability of identifying a specific pesticide. However, their sensitivity is sufficient to detect minimum level of pesticides imposed by regulatory agencies. In the future, AChE-based biosensors would complement or serve as an alternative to more expensive and complex chromatographic devices.

ACKOWLEDGEMENTS

Partial support of this work by the National Natural Science Foundation of China (No. 21305004) and the Joint Fund for Fostering Talents of National Natural Science Foundation of China and Henan Province (U1304205) is gratefully acknowledged.

References

1. M. B. Kralj, P. Trebše and M. Franko, *Trac-Trend. Anal. Chem.*, 26 (2007) 1020.
2. S. Pundir, N. Chauhan, J. Narang and C. S. Pundir, *Anal. Biochem.*, 427 (2012) 26.
3. W. Zhang, A. Mohamed Asiri, D. Liu, D. Du and Y. Lin, *Trac-Trend. Anal. Chem.*, 54 (2014) 1.
4. G. Liu and Y. Lin, *Anal. Chem.* , 78 (2006) 835.
5. L. Liu, Y. Gao, H. Liu, J. Du and N. Xia, *Electrochim. Acta*, 139 (2014) 323.
6. N. Xia, L. Liu, R. Wu, H. Liu, S.-J. Li and Y. Hao, *J. Electroanal. Chem.*, 731 (2014) 78.
7. P. Raghu, B. E. K. Swamy, T. M. Reddy, B. N. Chandrashekar and K. Reddaiah, *Bioelectrochemistry* 83 (2012) 19.
8. N. B. Oujji, I. Bakas, G. Istamboulié , I. Ait-Ichou, E. Ait-Addi, R. Rouillon and T. Noguer, *Food Control*, 30 (2013) 657.
9. B. Bucur, D. Fournier, A. Danet and J. L. Marty, *Anal. Chim. Acta*, 562 (2006) 115.
10. K. Anitha, S. V. Mohan and S. J. Reddy, *Biosens. Bioelectron.*, 20 (2004) 848.
11. P. Raghu, T. M. Reddy, B. E. K. Swamy, B. N. Chandrashekar, K. Reddaiah and M. Sreedhar, *J. Electroanal. Chem.*, 665 (2012) 76.
12. P. Raghu, T. M. Reddy, K. Reddaiah, B. E. K. Swamy and M. Sreedhar, *Food Chem.*, 142 (2014) 188.
13. M. Pohanka, J. Fusek, V. Adam and R. Kizek, *Int. J. Electrochem. Sci.*, 8 (2013) 71.
14. E. Suprun, G. Evtugyn, H. Budnikov, F. Ricci, D. Moscone and G. Palleschi, *Anal. Bioanal. Chem.*, 383 (2005) 597.
15. Y. Song, M. Zhang, L. Wang, L. Wan, X. Xiao, S. Ye and J. Wang, *Electrochim. Acta*, 56 (2011) 7267.
16. X. Sun and X. Wang, *Biosens. Bioelectron.*, 25 (2010) 2611.
17. J. Mukherjee and J. R. Kirchhoff, *Anal. Chem.*, 81 (2009) 6996.
18. R. K. Mishra, R. B. Dominguez, S. Bhand, R. Muñoz and J. L. Marty, *Biosens. Bioelectron.*, 32 (2012) 56.
19. C. Bonnet, S. Andreescu and J. L. Marty, *Anal. Chim. Acta*, 481 (2003) 209.
20. G. Istamboulie, T. Sikora, E. Jubete, E. Ochoteco, J. L. Marty and T. Noguer, *Talanta*, 82 (2010) 957.
21. L. G. Zamfir, L. Rotariu and C. Bala, *Biosens. Bioelectron.*, 46 (2013) 61.
22. R. Sinha, M. Ganesana, S. Andreescu and L. Stanciu, *Anal. Chim. Acta* 661 (2010) 195.
23. H. Zejli, J. L. H. H. Cisneros, I. Naranjo-Rodriguez, B. Liu, K. Temsamani and J. L. Marty, *Talanta*, 77 (2008) 217.
24. Y. Liu and M. Wei, *Food Control* 36 (2014) 49.
25. R. R. Dutta and P. Puzari, *Biosens. Bioelectron.*, 52 (2014) 166.

26. L. Asturias-Arribas, M. A. Alonso-Lomillo, O. Domínguez-Renedo and M. J. Arcos-Martínez, *Talanta*, 111 (2013) 8.
27. K. A. Law and S. P. J. Higson, *Biosens. Bioelectron.*, 20 (2005) 1914.
28. L. B. Hu, D. S. Hecht and G. Gruner, *Chem. Rew.*, 110 (2010) 579.
29. Y. Lin, F. Lu and J. Wang, *Electroanalysis*, 16 (2004) 145.
30. K. A. Joshi, J. Tang, R. Haddon, J. Wang, W. Chen and A. Mulchandani, *Electroanalysis*, 17 (2005) 54.
31. D. Du, X. Huang, J. Cai and A. Zhang, *Sensor. Actuat. B*, 127 (2007) 531.
32. D. Du, M. Wang, J. Cai, Y. Tao, H. Tu and A. Zhang, *Analyst*, 133 (2008) 1790.
33. D. Du, X. Huang, J. Cai, A. Zhang, J. Ding and S. Chen, *Anal. Bioanal. Chem.*, 387 (2007) 1059.
34. J. Dong, X. Fan, F. Qiao, S. Ai and H. Xin, *Anal. Chim. Acta*, 761 (2013) 78.
35. L. G. Zamfir, L. Rotariu and C. Bala, *Biosens. Bioelectron.*, 26 (2011) 3692.
36. A. N. Ivanov, R. R. Younusov, G. A. Evtugyn, F. Arduini, D. Moscone and G. Palleschi, *Talanta*, 85 (2011) 216.
37. H. Chen, X. Zuo, S. Su, Z. Tang, A. Wu, S. Song, D. Zhang and C. Fan, *Analyst*, 133 (2008) 1182.
38. F. C. Moraes, L. H. Mascaro, S. A. S. Machado and C. M. A. Brett, *Talanta* 79 (2009) 1406.
39. L. Rotariu, L. G. Zamfir and C. Bala, *Anal. Chim. Acta*, 748 (2012) 81.
40. D. Du, X. Ye, J. Cai, J. Liu and A. Zhang, *Biosens. Bioelectron.*, 25 (2010) 2503.
41. S. Çevik, S. Timur and Ü. Anik, *Electroanalysis*, 25 (2013) 2377.
42. S. P. Sharma, L. N. S. Tomar, J. Acharya, A. Chaturvedi, M. V. S. Suryanarayan and R. Jain, *Sensor. Actuat. B*, 166-167 (2012) 616.
43. S. Viswanathan, H. Radecka and J. Radecki, *Biosens. Bioelectron.*, 24 (2009) 2772.
44. J. W. Shen, Y. B. Li, H. S. Gu, F. Xia and X. L. Zuo, *Chem. Rew.*, 114 (2014) 7631.
45. L. Liu, Y. Xing, H. Zhang, R. Liu, H. Liu and N. Xia, *Int. J. Nanomed.*, 9 (2014) 2619.
46. Y. Xing, L. Liu, D. Zhao, Y. Yang and X. Chu, *Materials*, 7 (2014) 5554.
47. O. Shulga and J. R. Kirchhoff, *Electrochem. Commun.* , 9 (2007) 935.
48. D. Du, S. Chen, J. Cai and A. Zhang, *Biosens. Bioelectron.*, 23 (2007) 130.
49. D. Du, J. Ding, J. Cai and A. Zhang, *J. Electroanal. Chem.* , 605 (2007) 53.
50. H. Yin, S. Ai, J. Xu, W. Shi and L. Zhu, *J. Electroanal. Chem.*, 637 (2009) 21.
51. J. Gong, L. Wang and L. Zhang, *Biosens. Bioelectron.*, 24 (2009) 2285.
52. P. Norouzi, M. Pirali-Hamedani, M. R. Ganjali and F. Faridbod, *Int. J. Electrochem. Sci.*, 5 (2010) 1434.
53. Y. Qu, Q. Sun, F. Xiao, G. Shi and L. Jin, *Bioelectrochemistry* 77 (2010) 139.
54. S. Hou, Z. Ou, Q. Chen and B. Wu, *Biosens. Bioelectron.*, 33 (2012) 44.
55. N. Chauhan and C. S. Pundir, *Anal. Chim. Acta* 701 (2011) 66.
56. N. Chauhan and C. S. Pundir, *Electrochim. Acta* 67 (2012) 79.
57. L. Zhang, J. Chen, Y. Wang, L. Yu, J. Wang, H. Peng and J. Zhu, *Sensor. Actuat. B*, 193 (2014) 904.
58. D. Huo, Q. Li, Y. Zhang, C. Hou and Y. Lei, *Sensor. Actuat. B* 199 (2014) 410.
59. J. Ding, H. Zhang, F. Jia, W. Qin and D. Du, *Sensor. Actuat. B*, 199 (2014) 284.
60. C. Zhai , X. Sun , W. Zhao, Z. Gong and X. Wang, *Biosens. Bioelectron.*, 42 (2013) 124.
61. I. Cesarino, F. C. Moraes and S. A. S. Machado, *Electroanalysis*, 23 (2011) 2586.
62. I. Cesarino, F. C. Moraes, M. R. V. Lanza and S. A. S. Machado, *Food Chem.*, 135 (2012) 873.
63. A. Siriviriyanun, T. Imae and N. Nagatani, *Anal. Biochem.*, 443 (2013) 169.
64. A. Ambrosi, C. K. Chua, A. Bonanni and M. Pumera, *Chem. Rew.*, 114 (2014) 7150.
65. S. Wu, F. Huang, X. Lan, X. Wang, J. Wang and C. Meng, *Sensor. Actuat. B*, 177 (2013) 724.
66. Y. Li, Y. Bai, G. Han and M. Li, *Sensor. Actuat. B* 185 (2013) 706.
67. H. Zhang, Z. Li, A. Snyder , J. Xie and L. A. Stanciu, *Anal. Chim. Acta*, 827 (2014) 86.
68. T. Liu, H. Su, X. Qu, P. Ju, L. Cui and S. Ai, *Sensor. Actuat. B*, 160 (2011) 1255.
69. L. Zhang, L. Long, W. Zhang, D. Du and Y. Lin, *Electroanalysis*, 24 (2012) 1745.

70. Y. Yang, A. M. Asiri, D. Du and Y. Lin, *Analyst*, 139 (2014) 3055.
71. L. Yang, G. Wang and Y. Liu, *Anal. Biochem.*, 437 (2013) 144.
72. K. Wang, H. N. Li, J. Wu, C. Ju, J. J. Yan, Q. Liu and B. Qiu, *Analyst*, 136 (2011) 3349.
73. Q. Zhou, L. Yang, G. Wang and Y. Yang, *Biosens. Bioelectron.*, 49 (2013) 25.
74. G. Wang, X. Tanb, Q. Zhou, Y. Liu, M. Wang and L. Yang, *Sensor. Actuat. B*, 190 (2014) 730.
75. P. Nayak, B. Anbarasan and S. Ramaprabhu, *J. Phys. Chem. C*, 117 (2013) 13202.
76. Y. Wang, S. Zhang, D. Du, Y. Shao, Z. Li, J. Wang, M. H. Engelhard, J. Li and Y. Lin, *J. Mater. Chem.*, 21 (2011) 5319.