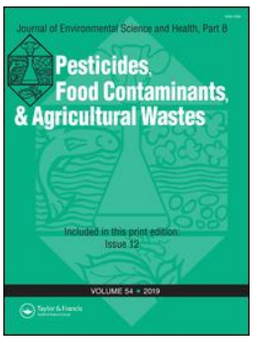

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REVIEW ARTICLE

Development of enzymatic electrochemical biosensors for organophosphorus pesticide detection

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ABSTRACT

The enzymatic electrochemical biosensor has the advantages of simple operation, speed, and integration in the detection of organophosphorus pesticide (OPs) residues. It has the potential to become the best alternative to the traditional OP detection technology. This article introduces the OP identification principle of different enzymes, the OP detection mechanism of several common sensors, and the enzyme assembly method. In addition, the article discusses application of nanomaterials in sensor preparation and sensor performance parameters in the past decade. The related content of early sensors is outside the scope of this article.

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organophosphorus pesticides; cholinesterase; electrochemical sensor; carrier material

Introduction

As global warming intensifies, crop diseases and insect pests are becoming increasingly severe,[1] which results in the widespread use of pesticides in agricultural production. As a large agricultural country, China has seen an increasing trend in pesticide use since the 1990s. In 1991, the use of pesticides in China reached 765,300 tons, and in 2013 it increased to 1.8019 million tons, ranking first in the world. Organophosphorus pesticides (OPs), including dichlorvos (DDVP), dimethoate, parathion, and trichlorfon (the structure is shown in Fig. 1), are the most widely used pesticides, accounting for 60–70% of the pesticides that cause human poisoning.[2] While these pesticides protect against pests and diseases, the residual problems caused by their utilization are very critical. According to observations, only about 10% of powdered pesticide adheres to plants when sprayed in the field; likewise, only 20–30% of liquid pesticides adheres to plants.[3]

Organophosphorus pesticide remains on the surface of vegetables and fruits, or accumulate in livestock, fish and shrimp through the food chain. Human who consume fruits or vegetables with OPs residues may become poisoned. High levels of OPs can damage the human nervous system, leading to acute poisoning symptoms such as convulsions, dyspnea, arrhythmia, muscle twitching, and hypoxia; Low levels of OPs can also cause chronic poisoning resulting in damage to the heart, liver, kidneys and other organs.[4] Acetylcholinesterase (AChE) catalyzes the hydrolysis of acetylcholine (ATCl). The phosphate group in OPs binds to the protein molecule AChE in the human body to phosphorylate the enzyme.[5] This mechanism is illustrated in Figure 2. The enzyme activity is inhibited and the rate of the enzyme-catalyzed decomposition of ATCl is reduced. Taking brain nerve cells as an example, ATCl as a neurotransmitter, is released by the presynaptic membrane and acts on the post-synaptic membrane, causing nerve excitement, thereby achieving the information transmission. Under normal physiological conditions, ATCl will be decomposed by AChE, but because OPs inhibit the enzyme activity, ATCl continuously stimulates the post-synaptic membrane, causing sustained nerve excitement.[6] This causes clinical symptoms such as foaming in the mouth[7] and muscle twitching.

Organophosphorus pesticide residues can seriously threaten people's lives, health, and safety, thus, countries and regions such as China and the European Union have formulated relevant maximum residue standards laws and regulations. Table 1 presents some standards for the maximum residue level of pesticides on strawberry surfaces established by China and the European Union.[8]

The increasing awareness of the safety of agricultural products, it has prompted the continuous development of pesticide detection technologies. In the late 1950s, researchers began to explore OP detection technologies. Initially, chemical methods, colorimetric methods, and volumetric methods were used to detect OP residues. These methods have low sensitivity. In the late twentieth century, with the development of chromatography, researchers began using thin layer chromatography. Gas Chromatography/Solid Phase Chromatography,[9,10] and High Performance Gas Chromatography[11] have led to a new level OP detection and are still in use today. Chromatography has the characteristics of good detection sensitivity, accurate detection, precision and low cost.[12] However, the detection process is time and generates a large amount of organic solvent

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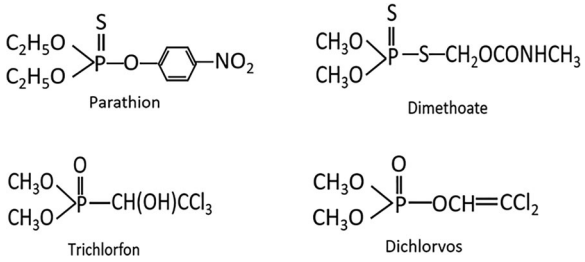

Figure 1. The chemical structure of four kinds of OPs.

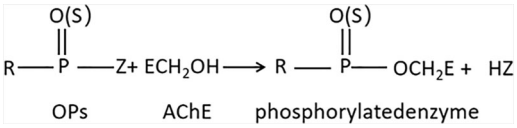

Figure 2. Reaction between AChE and Ops.

Table 1. Strawberry pesticide MRLs standard (mg kg^{-1}).

Pesticide	China	EU
Dichlorvos	0.2	0.1
Dibendazim	0.1	0.05
Chlorpyrifos	—	0.05
Dimethoate	0.5	0.02
Chlorothalonil	1.0	3.0
Fenthion	0.05	0.05
DDT	0.05	0.05
Parathion	cannot be detected (LOD < 0.01)	0.05
Carbendazim	0.5	0.1
Phorate	cannot be detected (LOD < 0.001)	0.05
Malathion	1.0	0.5
Phoxim	0.5	—
Acephate	0.5	0.02

waste;[13] thus, this method is difficult to popularize and use for detection involving large quantities.

The enzyme-based electrochemical biosensor has the advantages of simple operation, short detection period, timely detection, and low cost, and it meets the market needs. Table 2 lists the structure and detection performance parameters of some AChE sensors. Presently, the technology related to the use of electrochemical biosensors for OP detection is not mature enough, nonetheless, the number of researchers, amount of research funding and the number of published papers in this field have exponentially increased in recent years.[23]

Principle and mechanism of OP identification

The enzymes used in the research on OPs sensors include cholinesterase, biohydrolase, and plant esterase.[24] Plant esterases can be extracted from wheat flour, and the extraction steps refer to Yang.[25] However, most studies mainly consider cholinesterase and biohydrolase. The former includes AChE and butyrylcholinesterase (BChE). As shown in Figure 3a, AChE catalyze the decomposition of its natural substrate, ATCl, to produce choline and acetic acid. The principle of BChE is similar to that of AChE. Generally, BChE is more sensitive to OPs. In theory, sensors constructed with butyrylcholinesterase have higher detection accuracy. In addition, as presented in Table 3,[26]different cholinesterases have different sensitivities to the same kind of OPs, and the inhibitory effects of different kinds of OPs on the same enzyme are also very different. Even the same enzymes from different sources are inhibited to different degrees by the same OP concentration.

As shown in Figure 3b, OPs can be hydrolyzed by ophosphate hydrolase (OPH) (phosphatase, carboxylesterase, amide hydrolase) to produce ethanol, hydrogen ions, or other products with electroactive, luminescent groups. These products can be converted into electrical or physical signals. However, there is currently no way to synthesize large amounts of hydrolytic enzymes by artificial methods, which limits the development and application of this hydrolytic enzyme-based technology. The extraction technology of high-purity AChE is mature and can reach a commercial scale. The related research is earlier than the research on organophosphorus hydrolase. Therefore, the depth and breadth of research on AChE sensors are higher than those of the research on other types of cholinesterase and biohydrolase.

Biosensor for detecting organic phosphorus pesticide

Electrochemical biosensors use solid electrodes as basic electrodes to fix bio-sensitive molecules on the electrode surface. Through the specific recognition between biomolecules, biological signals and chemical signals are converted into physical or electrical signals that can be identified easily and measured. This method has the advantages of simple operation, compact equipment, and rapid detection, and has broad application prospects in the field of organic phosphorus pesticide detection. Biosensors for detecting OPs include potentiometric electrochemical sensors,[27–29] ion field sensitive field effect tubes,[30,31] amperometric electrochemical sensors and photoelectric electrochemical sensors. Potential type sensors and ion field sensitive effect tubes have been reported previously. The detection accuracy of this type of sensor for OPs is not high, at the μM level, and the detection steps are relatively complicated generally. Therefore, current-type and photoelectric-type electrochemical biosensors have become research hotspots in recent years.

Current type electrochemical sensor

Among the electrochemical sensors that detect OPs, current-type electrochemical sensors account for the majority. Enzymes are generally assembled on the surface of inert electrodes, such as graphite electrodes, glassy carbon (GC) electrodes, precious metal electrodes, and printed electrodes. The enzyme catalyzes the choline production by its substrate, and the current signal formed by the electrons produced by the anodic oxidation of choline can be detected. Generally, the chemical signals are converted into current or voltage signals through electrochemical characterization methods such as cyclic voltammetry (CV), amperometric, differential pulse voltammetry (DPV), and square-wave voltammetry (SWV). Given that the intensity of the current (voltage) signals before and after the enzyme inhibition reflect the enzyme inhibition rate, the inhibition rate can be used as

Table 2. Comparison of the analytical characteristics of the AChE biosensor for OPs detection.

Sensor structure	Detection interval (nM)	LOD (nM)	Analyte	Reference
AChE-e-pGON/GCE	0.3–6.1	0.15	Carbar	Li Y et al.[14]
AChE/Fe_3O_4-CH/GCE	5–90	3.6	Carbofuran	Jeyapragasam and Saraswathi[15]
AChE-AuNPs/MPS/Au	3–200	1.0	Carbamate	Song et al.[16]
AChE/CS/Fe_3O_4	0.5–20	0.3	Malathion	Rodrigues et al.[17]
AChE/(Cs/ALB)s/GCE	250–1,500	3.9	DDVP	Guan H et al.[18]
AChE/Er-GRO–Nafion/GCE	22.6–450	9.1	DDVP	Wu et al.[19]
AChE/AuMWNTs/Au	0.1–7	1	Paraoxon	Jha and Ramaprabhu[20]
SH-β-CD^h/AuNPs/SWNTs/GCE	2–80	0.1	Parathion-methyl	Yotova and Medhat[21]
AChE-CS/TiO_2/GCE	1.13–22,600	0.23	DDVP	Hu et al.[22]

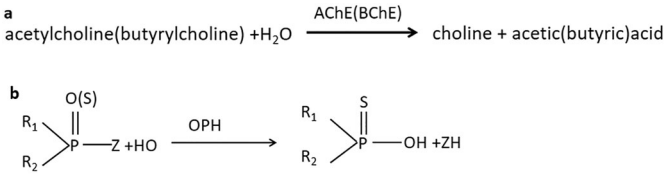

Figure 3. Reaction scheme for the AChE/BChE-catalyzed hydrolysis of ATCl/BTCl (a) and OPH-catalyzed hydrolysis of OPs (b).

Table 3. AChE inhibition rate is 50% corresponding to various organophosphorus pesticide concentrations ($\mu g\ mL^{-1}$).

Pesticide	AChE comes from the Italian bee	AChE comes from mice
Dichlorvos	0.01626	0.0464
Monocrotophos	1.136	0.9320
Chlorpyrifosmethyl	1.232	7.053
Dimethoate	1.257	54.957
Diazinon	3.199	0.0201
Methamidophos	4.456	23.209
Acephate	7.089	5.939
Parathion	1.0009	3.879
Phorate	0.6734	2.953
Malathion	0.6221	0.5720
Acephate	4.296	91.965

the OP concentration information. As shown in Figure 4, Wang[32] used silver nanowires to modify rGO and applied it to the surface of the glassy carbon electrode (GCE), and then assembled titanium oxide sol gel, chitosan (CS) and AChE in turn. rGO could effectively increase the carrier surface area, and silver nanowires could connect the gaps between rGO nano-debris, thereby improving the sensor conductivity. The current signal of this sensor decreased with increasing DDVP content. The detection limit was 7.4 nM in the DDVP concentration range of 36 nM–22.63 µM. Jha[20] used nano-gold and carbon nanotube modified enzymes on the gold electrode. The sensor had good conductivity and the detection limits for oxygen and phosphorus were as low as 1 nM. Jing[33] adopted three-dimensional graphene and copper oxide nanoflowers as the enzyme carrier. The structure had a large specific surface area and the sensor could detect 1 pM marathon. Zhang[34] used DNA and carbon nanotubes stacked in layers, which amplified the electrical signal and could detect OPs and non-OPs pesticides.

Photoelectric electrochemical sensor

The research on photoelectric electrochemical sensors for detecting OP detection has also received extensive attention. These sensors have been based on cholinesterase early catalyzing the substrate hydrolysis, causing the solution pH to drop. Owing to the effect of a color indicator, the pH is outputted as a light signal. Andres[35] covalently fixed AChE on isothiocyanate glass beads, and the indicator thymol blue was fixed on nitropropyl glass beads by a condensation reaction with formaldehyde to realize OP detection by optical-electrochemical biosensors. When AChE hydrolyzes acetylcholine chloride, the pH of the environment decreased, changing the color of the thymol blue indicator from blue-green to yellow. This change led to an increase in the reflected optical density, and the weak pH change signal was amplified by the color change. The sensor could detect paraoxon of 27.1 $\mu g \cdot L^{-1}$. The detection accuracy of sensors prepared by the principle of indicator color development is generally low. In recent years, sensors that convert chemical signals into optical signals have been developed based on the quantum-dot quenching principle, and these sensors have improved detection accuracy. As shown in Figure 5, Zheng[36] adopted cadmium telluride quantum and organic polymers as enzyme carriers to prepare sensors on a glass substrate. Cholinesterase catalyzed the substrate hydrolysis to produce choline. Quantum dots emitted light under ultraviolet radiation. Choline (TCl) could quench luminescent quantum dots. According to the intensity of the radiation spectrum before and after inhibition, the enzyme inhibition rate was calculated, and the detection limits for paraoxon and parathion were 10.5 pM and 4.47 pM, respectively. Sahub[37] built an organic phosphorus pesticide detection system based on choline oxidase, AChE and graphene quantum dots. Choline oxidase oxidize choline and produce hydrogen peroxide, which quench the photoluminescence of graphene quantum dots. The detection limit of this system for DDVP was 0.778 µM. Ban[38] used manganese-doped zinc sulfide quantum dots combined with precious metal nanowires as the enzyme carrier, and the detection limits for oxygen and phosphorus were as low as 0.1 pM. Although photoelectric electrochemical sensors have accurate detection and high sensitivity, they rely on luminescence spectrometers, absorption spectrometers and other instruments to emit and receive spectral signals of special frequencies. Since the detection of optical signals relies on expensive equipment and the detection is easily disturbed by the environment, amperometric electrochemical sensors have advantages in large-scale promotion.

Enzyme immobilization method

The catalytic active layer of the enzymatic electrochemical sensor for organic phosphorus-pesticide detection determines the sensor selectivity, sensitivity, stability, detection range, and service life. Traditional enzyme adsorption

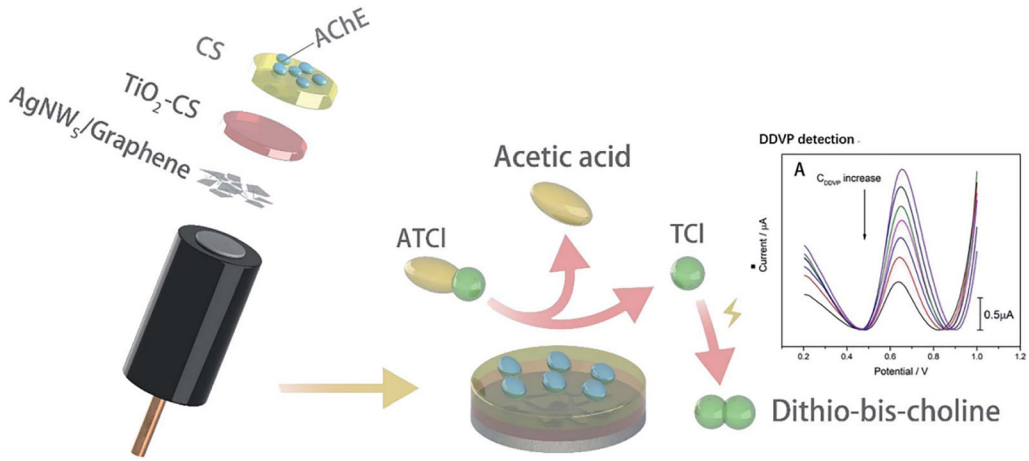

Figure 4. Schematic illustration of current electrochemical organophosphorus pesticide sensor structure and detection principle.

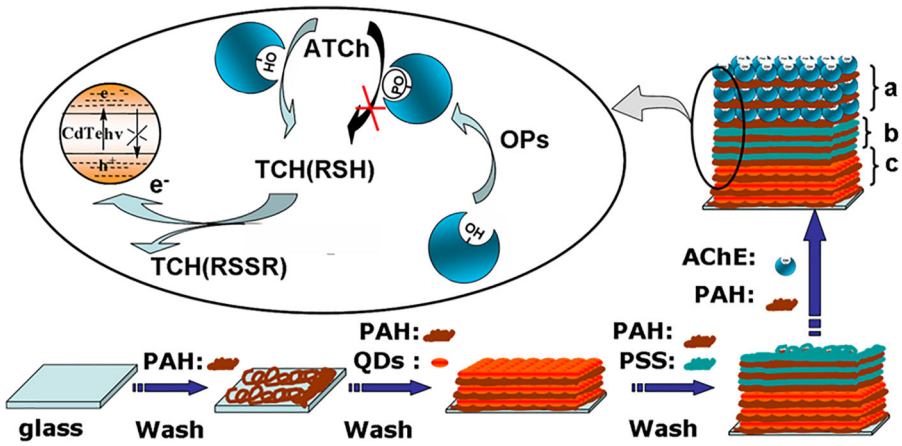

Figure 5. Schematic illustration of photoelectric electrochemical OPs sensor structure and detection principle.

include physical adsorption, embedding, and covalent bond coupling. Combining multiple fixing methods can yield better results. Table 4 lists the enzyme assembly method of the organophosphate pesticide sensor, and parameter information such as storage stability time.

Physical adsorption method

The physical adsorption method is earliest and the simplest enzyme immobilization method. The enzyme is immobilized on the carrier through van der Waals force, electrostatic force, hydrogen bonding force and ionic bond. Cui[48] fixed negatively charged enzymes on the surface of positively charged CS. Owing to the interaction of electrostatic forces, the sensor exhibited good repeatability and stability. Rui[49] synthesized the aminated mesoporous material SBA-15 as the enzyme carrier. The enzyme assembly was realized through $NH3^{+}$ adsorbed on the SBA-15 molecule and the $-COO^{-}$ charged group in the enzyme molecule.

Embedding method

The embedding method involves entrapping the enzyme in the carrier, and the most common methods include sol-gel embedding and polymer embedding. The carrier of the lyogel embedding method has good biological compatibility, stable physical and chemical properties, and certain flexibility. The carrier usually has a porous structure, and the pores restrict the penetration of macromolecules such as enzymes, but allow the penetration of small molecules such as ATCl. The most common sol-gel carriers are metal alkoxy compounds, which undergo hydrolytic condensation with water to form sols. As the water evaporates, a gel net cage structure is formed. Titanium oxide sol gel is also a common coated carrier material. Cui[48] used titanium oxide sol gel as a carrier. Enzymes and CS penetrate the titanium oxide and are embedded. The sensor shows good stability and repeatability. The polymer embedding method is common for multilayer sandwich structures. Zheng[36] adopted multilayer sandwich structures PAH/AChE/PAH, and PAH/ChOx/PAH as the catalytic active layers and polycyclic aromatic hydrocarbons (PAH) as the porous organic polymer. Acetylcholine and choline freely penetrate while effectively blocking the diffusion of enzyme protein macromolecules. Zhang[50] used the polymer NHS/EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) and the conductive polymer FBThF (4,7-di(furan-2-yl)benzo[c][1,2,5]thiadiazole) to oat AChE to fabricate an OP pesticide. The biocatalytic activity of the sensor hardly decreased within 10 days. In recent years, there have also

Table 4. Various AChE inhibition based amperometric biosensors fabricated for OP detection.

Electrode structure	Immobilization method	Analyte	Incubation time (min)	Storage stabiliety (day)	Reference
SWCNT-CoPC/SPE	Covalent	Paraoxon/Malaoxon	15	3	Ivanov et al.[39]
MWCNT-Au nano /GCE	Adsorption	Malathion	8	30	Ivanov et al.[40]
TiO_2@grephene/GCE	Adsorption	Cabaryl	3	20	Wang et al.[41]
AuNPs-$CaCO_3$/Au	Adsorption	Malathion/Chlorpyrifos	10	90	Chauhan et al.[42]
PVA-SbQ membrane/Pt	Entrapment	Paraoxon/Maneb	30	30	Marty et al.[43]
PAMAM-Au/CNTs/GCE	Electrostatic	Carbofuran	9	21	Qu et al.[44]
Silica sol-gel/SPE	Encapsulation	Paraoxon/ Dichlorvos	20	6	Andreescu et al.[45]
PB and CHIT/GCE	Glutaraldehyde/Crosslinking	Dichlorvos/Omethoate	10	NR	Sun and Wang[46]
ZrO_2/CHIT/GCE	Adsorption	Phoxim/Malathion	15	30	Yang et al.[47]

been reports on the method of using metal nano-mesh[51] to wrap the immobilized enzyme.

Crosslinking method

The crosslinking method involves the use of double or multi-functional crosslinking agents to form crosslinks between the enzyme molecules to achieve enzyme immobilization. Since the enzyme molecule and the crosslinking agent form a water-insoluble three-dimensional crosslinked body, which easily adheres to the solid surface, the crosslinking method is commonly used in preparing enzyme electrodes. Ivanonc[28] used glutaraldehyde to crosslink BChE and nitrocellulose to form a thin film and assembled the film on the surface of a flat glass pH electrode. The detection limit of the sensor for trichlorfon was 0.01 g L^{-1}. Vakurov[52] adopted aniline to activate printed carbon electrode and glutaraldehyde as a crosslinking agent to fix the enzyme onto the electrode surface. In addition to glutaraldehyde, EDC/NHS, a common crosslinking agent, is also used for enzyme immobilization.[53,54]

Covalent bond coupling method

The covalent bond coupling method involves the activation of the graphite electrode and the glassy carbon electrode to attach functional groups such as carbonyl, carboxyl, and amino groups to their surfaces, then, the electrodes react with the carboxyl and amino groups in the enzyme molecule to develop a covalent bond. Because the covalent bond is relatively strong, the binding between the enzyme and the carrier is tight, but due to the organic chemical reaction involved, the operation is complicated and the reaction conditions are difficult to control. Guler[55] modified aminated rGO with silver nanoparticles, and immobilized the enzyme through a covalent bond. Song[56] immobilized enzymes on the surface of amidated carbon nanotubes. The carboxylic acid group in the enzyme molecule dehydrates and condenses with the amino group on the surface of the carbon nanotubes to form a covalent bond. The sensor detection limits for monocrotophos and chlorpyrifos were 0.2 pM and 3 pM, respectively. Under ultraviolet irradiation, Jiang[57] immobilized the enzyme by covalently coupling diazo resin to the enzyme. The detection limits of this sensor for malathion and methyl parathion were as low as 0.0022 pM and 0.0016 pM, respectively.

Enzyme carrier construction

The carrier one of the important structures for constructing the sensor, determines the firmness of the sensor structure and the enzyme layer catalytic activity, thereby affecting the sensor sensitivity, service life, and detection range. In the past 10 years, the research on the types and structures of materials for constructing enzyme carriers has become a hot spot hotspot in the research on OP sensors, and various biomimetic materials with good biocompatibility have been applied for the OP sensor fabrication. Enzyme carrier materials must have the following characteristics. First, they must have good biocompatibility while maintaining the enzyme's good catalytic activity without destroying the enzyme structure. Second, they must have a large specific surface area, which is conducive to the assembly of enzymes and the improvement of the enzyme catalytic efficiency. Third the physical and chemical properties must be stable to ensure the sensor stability. These materials include sol-gels, carbon nanomaterials, transition metal oxides in various forms, and high molecular polymers. Metal nanomaterials can be used as carriers for enzymes or to modify biocompatible carrier materials with poor conductivity to improve the sensor conductivity.

Organic phosphorus pesticide sensor based on sol-gel carrier

Cui[48] used the sol-gel method to synthesize titanium sol-gel to prepare an organic phosphorus pesticide sensor. As shown in Figure 6A, the thin film layer formed after the titanium oxide sol-gel was dried exhibited particles with 6.3 nm diameter and holes with 6.5 nm diameter. After a small amount of CS was added, the film flexibility could be enhanced. Moreover, Figure 6B shows that the film surface significantly increased, which is conducive to the enzyme adhesion. The detection range of this sensor for DDVP was 36 nM–22.6 μM, and the detection limit was 29 nM. The carrier titanium oxide carrier of the sensor belongs to semiconductor and chitosan which are not conductive. The conductivity of the sensor is further optimized by the use of metal nanomaterials. In the subsequent study, Cui[58] adopted mesoporous silica (MS) to coat gold nanorods doped with titanium oxide sol-gel. The sensor structure is shown in Figure 6C. As shown in Figure 6D, in the DPV characterization, the peak current of the AuNRs@MS-doped titanium oxide sol-gel was significantly stronger than that of the undoped sol-gel. The electrical impedance spectroscopy

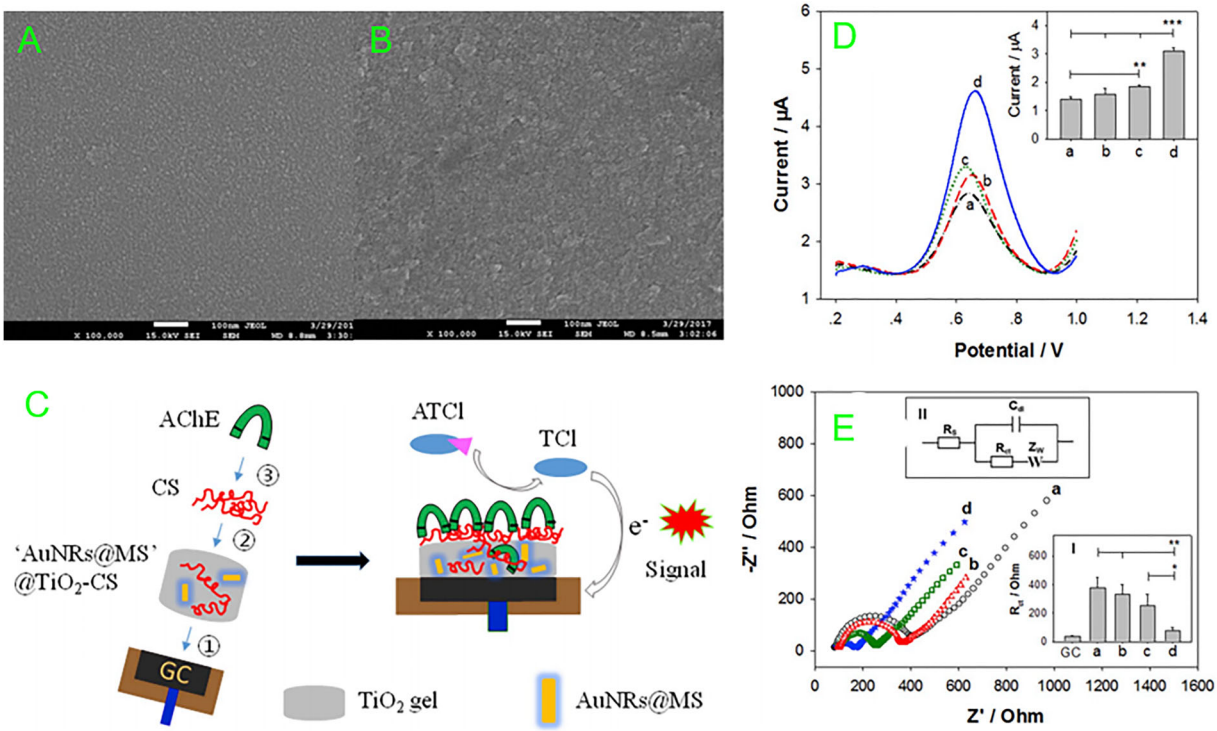

Figure 6. (A) SEM image of TiO_2 sol-gel film. (B) SEM image of TiO_2 sol-gel film with doping concentration of 0.005% CS. (C) Schematic illustration of sensor structure.(D) DPV curves of (a) AChE/CS/H_2O@ TiO_2-CS/GC, (b) AChE/CS/TiO_2-CS/GC, (c) AChE/CS/AuNRs@ TiO_2-CS/GC, and (d) AChE/CS/AuNRs@ MS@ TiO_2-CS/GC electrodes in PBS containing 1 mM ATCl. Inset in A: the corresponding statistic Icat values of the DPV curves.(E) The EIS of (a) H_2O @ TiO_2-CS/GC, (b) TiO_2-CS/GC, (c) AuNRs@ TiO_2-CS/GC, and (d) AuNRs@ MS@ TiO_2-CS/GC electrodes in $[Fe(CN)6]^{3-/4-}$ (10/10 mM). Inset I in B: the corresponding statistic Rct values obtained from the EIS spectra. Inset II in B: the Randles equivalent circuit model.

result in Figure 6E shows that the impedance of TiO_2-CS/GC was 380 Ω and that of AuNRs@MS-TiO_2-CS/GC was 77 Ω. The improved conductivity of the sensor improved the detection sensitivity. The detection limit for DDVP was as low as 5.3 nM.

Organic phosphorus pesticide sensor based on carbon material carrier

Carbon nanomaterials include zero-dimensional carbon nanoparticles, one-dimensional carbon nanotubes, and two-dimensional graphene. As shown in Figure 7A, Wei[59] used gold nanoparticles modified carbon nanospheres (Cs) as an enzyme carrier, which improved the enzyme immobilization efficiency. The detection limits for methyl parathion and chlorpyrifos were 0.49 pM and 0.149 pM. Li[60] synthesized holey graphene oxide (HGO), whose morphology is shown in Figure 7B. The enzyme could be firmly attached to the HGO rough surface due to the existence of holds, and the conductivity was higher than that of ordinary graphene oxide (GO). HGO has good water solubility, substrate affinity, and good mass transfer capacity. It can be used not only as an enzyme immobilization reagent, but also as a biosensing platform for OP detection. Martin[61] adopted the Hummers method to synthesize rGO and used it as a carrier to prepare a simple structure for detecting carbaryl pesticides in food. Li[14] synthesized an electrochemically induced mesoporous graphene network (e-PGON) to construct an enzyme carrier. As Figure 7D, the GO was internally interstaggered. This structure could enable the conductivity of GO of different layers, and the electrochemical impedance of the staggered GO was significantly lower than that of ordinary GO. Dhull[62] adopted multi-layer carbon nanotubes to modify a gold electrode, and then assembled a layer of carbonyl-activated single-layer carbon nanotubes as an enzyme carrier. The good conductivity of carbon nanotubes improved the sensor sensitivity. The covalent bond coupling of enzyme and single-walled carbon nanotube the sensor stability. This biosensor had a minimum detection limit of 1.9, 2.3, 2.2, and 2.5 nM for methyl parathion, depot, chlorpyrifos, and endosulfan, respectively, in the detection range of 0.1–130 μM. The biosensor had good reusability (reusable for 55 times), and stable storage for two months.

Organic phosphorus pesticide sensor based on transition metal oxide carrier

Transition metal oxides are often used to construct gas-sensitive sensors for the detection of toxic or flammable and explosive gases such as nitrogen oxides, formaldehyde, and industrial exhaust gases. Because of their good biocompatibility, rich and regular morphology, and large specific surface area (Fig. 8 shows the morphology of eight transition metal oxides), they are ideal enzyme carriers for OP sensor preparation. Rodrigues[17] assembled CS-modified Fe_3O_4 magnetic nanoparticles on the surface of the printed carbon electrode. The magnetic nanoparticles with adhered CS had a strong structure, and the rough surface was conducive to the enzyme fixation. Khan[63] used tin oxide, cadmium-doped tin oxide and nickel-doped tin oxide as the enzyme

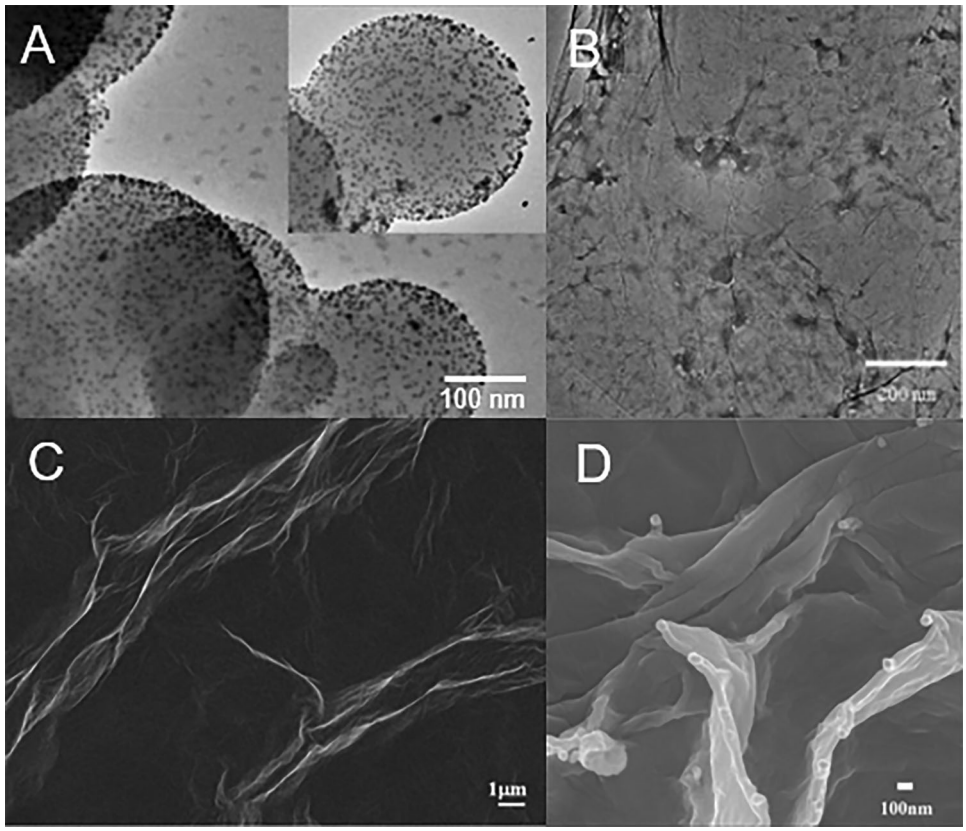

Figure 7. (A) SEM image of CS@Nano-Au(A), HGO(B), rGO(C) and e-PGON(D).

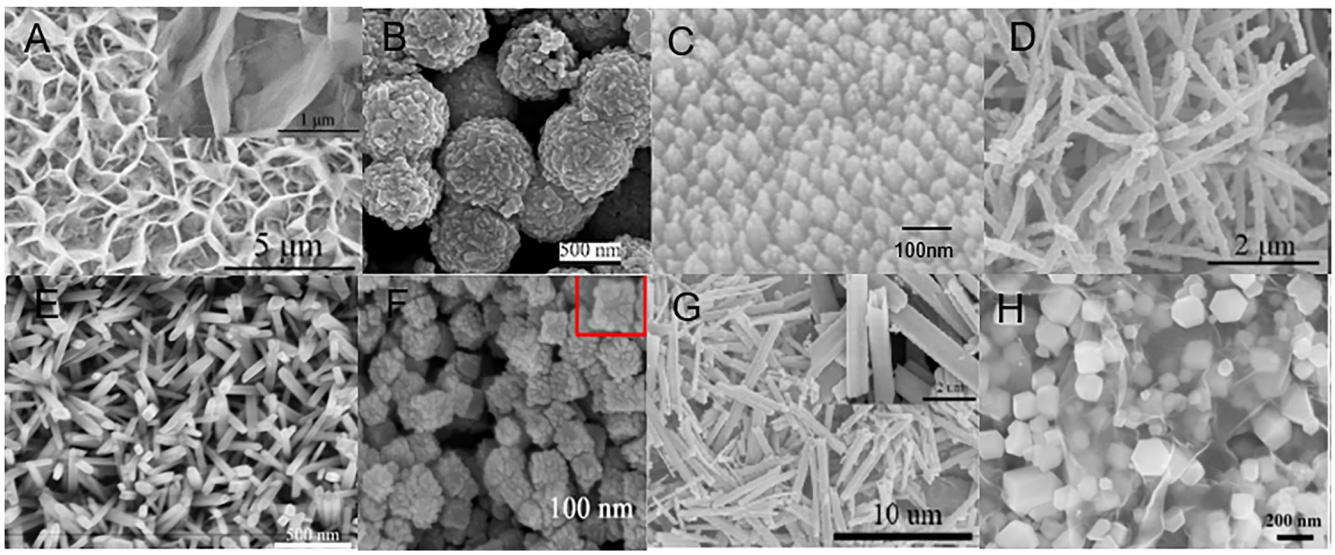

Figure 8. SEM image of NiO nano well (A), γ-Bi_2MoO_6 with Walnut shape (B), Porous Fe_2O_3 nano array (C), MoO_3 nanoflower (D), TiO_2 nano array (E), Fe_2O_3 nano cage (F), Mesoporous SnO_2 nano tubes (G) and hc-NiO/N-rGO (H). (Photo source of A–H: Heilongjiang University, Xu Yingming, the 14th National (China) Conference on Chemical Sensors in August 2019.).

carrier to modify a printed electrode. The detection limits for chlorpyrifos were 0.23 nM, 4.5 nM and 0.8 nM, respectively. Navin[5] tested the biocompatibility of the ZrO_2/RGO matrix, and the results showed that the material did not interfere with the normal physiological activities of living cells. Subsequently, a layer of ZrO_2/RGO composite structure was applied to the ITO surface as an enzyme carrier to prepare an organophosphorus pesticide sensor. The detection limit for chlorpyrifos was 0.1 pM–100 μM.

Organic phosphorus pesticide sensor based on precious metal nanomaterial carrier

Gold, silver, platinum, and other precious metal nanomaterials have excellent electrical conductivity and good biocompatibility. They are generally used to modify carriers and can also serve as carriers. Ju[64] prepared coral-shaped nano-gold materials. A layer of coral-shaped nano-gold materials was dripped on the RGO surface (Fig. 9A). The uneven

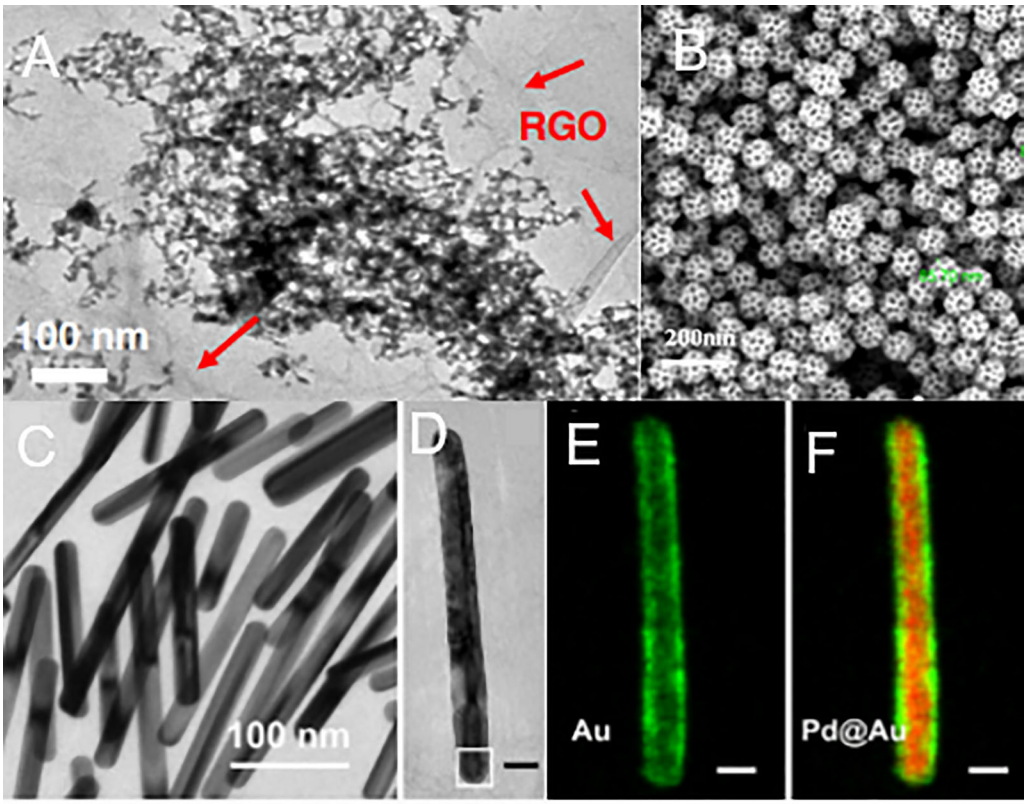

Figure 9. SEM image of Coral-like nano-Au (A), PdPt nano flowers (B), Pd@Au nanorods (C and D). Mapping image of Au nanorods (E) and Pd@Au nanorods (F).

structure of holes can accommodate and fix the enzyme molecules. Jiang[65] synthesized a GC electrode modified with PdPt nanoflower material (Fig. 9B). The diameter of the metal nano-flower was about 60–78 nm, the gap between the nano "petals" can aid in fixing the enzyme. The sensor had a detection limit of 1.7 pM for paraoxon in the range of 47 pM–47 nM. Lu[66] prepared Pd@Au nanomaterials with core-shell morphology. The surface (Fig. 9C and 9D) showed a uniform rod-like structure with regular size. The mapping characterization (Fig. 9E and 9F) showed that the nanorod outer layer was Au and the inner layer was Pd. The sensor could detect paraoxon pesticide residues of 3.6 pM. Ying[67] used CS-bonded silver nanowires as an enzyme carrier. Compared with irregularly arranged nanoparticles or nanorods, the nanowires arrangement could transfer electrons directionally and quickly. Chauhan[68] modified and assembled a layer of gold nanoparticles on $CaCO_3$ surface as an enzyme carrier. The gold nanoparticles could effectively improve the sensor conductivity. The sensor could detect various OP residues with concentrations ranging from 0.1 to 100 nM, including Malathion, and chlorpyrifos.

New two-dimensional nanomaterial carrier sensor based on titanium carbide, and molybdenum sulfide

The MXene $Ti_3C_2T_x$ belongs to transition metal carbides (T denotes -OH, -O, -F and other groups). The physical and chemical properties of this newly discovered two-dimensional material are similar to those of transition metal oxides such as good biocompatibility, and relatively large specific surface area.[69] Zhou[70] used HF to selectively etch aluminum in Ti_3AlC_2 to prepare the layered structure $Ti_3C_2T_x$ (Fig. 10A). The appearance of MXenes is similar to that of exfoliated graphite, and the average thickness of each layer is about 50 nm. The conductivity between the MXene layers is poor, and Jiang[71] used Ag nanoparticles to modify an MXene as an enzyme carrier. As shown in Figure 10B, Ag nanoparticles could be embedded in the gap between the MXene layer and the layer, thereby effectively improving the electron transmission performance of the sensor. The sensor could detect 0.01 pM marathon. The two-dimensional graphene-like material MoS_2 also has the advantages of large specific surface area, good biocompatibility, easy modification of molecular groups, sensitive surface state, and high electrocatalytic activity, which gives the material a broad application prospect in the field of electrochemical sensors.[72–75] However, the material has inadequate conductivity, which can be improved by doping or modification modifying with conductive nanomaterials. As shown in Figure 10C, Song[56] adopted a mild one-step thermal decomposition method[76] to synthesize N, F co-doped ultrathin two-dimensional MoS_2 (N-F-MoS_2). The N-F-MoS_2 had only a few layers and a thickness of only 3–5 nm. As shown in Figure 10D, Ag-nanoparticle-modified N-F-MoS_2 did not cause Ag agglomeration, and evenly adhered to the N-F-MoS_2 surface. The CV characterization (Fig. 10E) of the different structures of the sensor showed that the silver nanoparticles can effectively improve the sensor conductivity, and the sensor detection limit for chlorpyrifos was 3 pM.

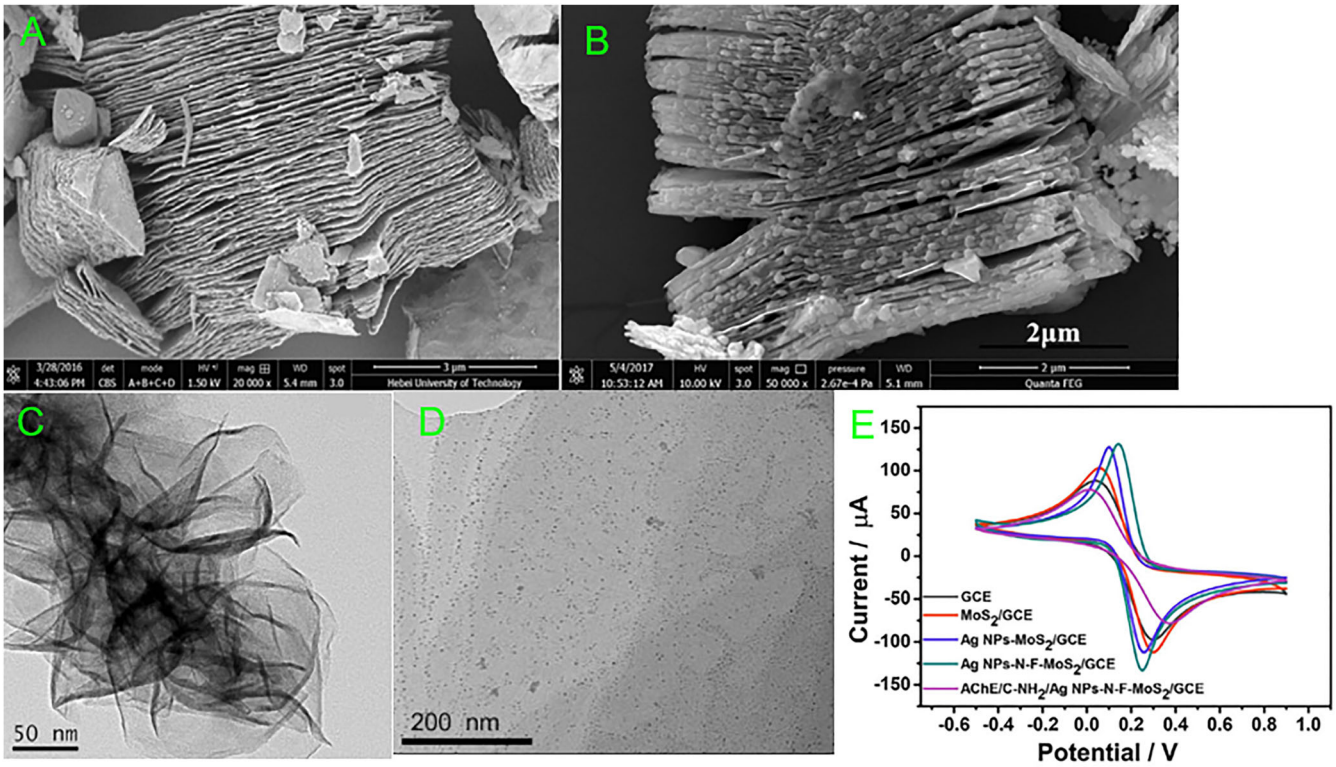

Figure 10. SEM image of $Ti_3C_2T_x$ (A), Nano Ag@$Ti_3C_2T_x$ (B), N-F-MoS_2 (C), Nano Ag@N-F-MoS_2 (D) and CV characterization of different sensor structures (E).

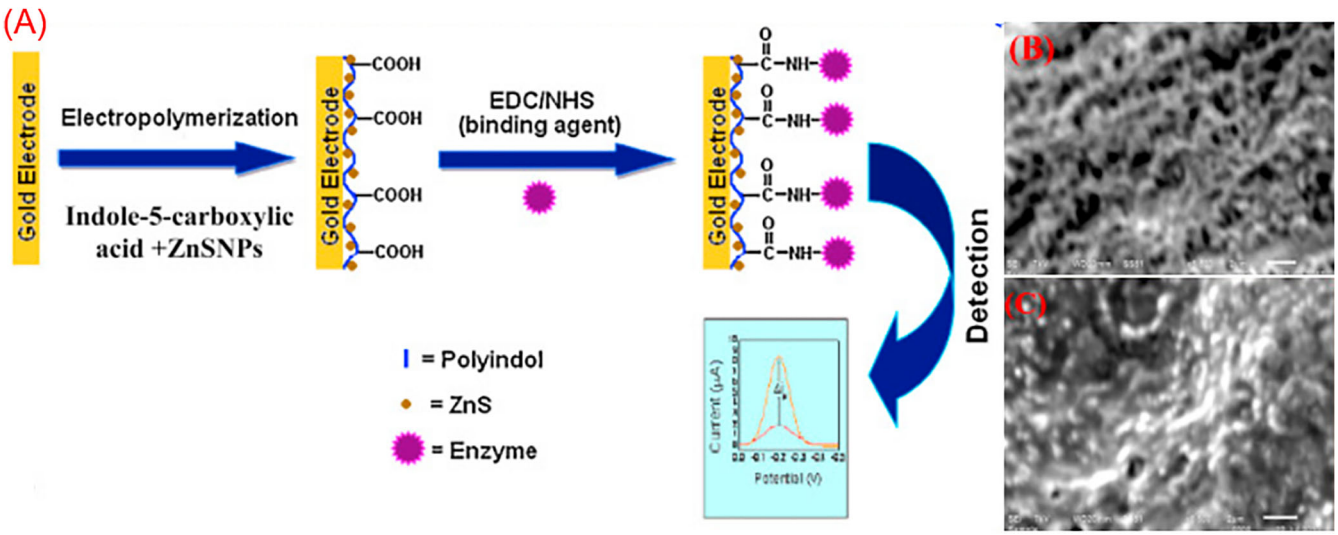

Figure 11. Schematic illustration of the biosensor fabrication and enzyme immobilization (A). SEM image of Pin5COOH/ZnS/AuEand (B) and AChE-Pin5COOH/ZnS/AuE (C).

Organic phosphorus pesticide sensor based on polymer material carrier

Polymer materials play a significant role in biosensor development due to their synthetic flexibility, structural presetting and diverse properties. Conductive electropolymers are usually used as electrodes or modified electrode materials, while non-conductive polymers are used as insulating materials, but are mainly applied as carriers for enzyme immobilization. High molecular polymers include conductive and non-conductive polymers. The conductive high molecular polymer is generally called synthetic metal[77] because of its excellent conductivity. Electrical polymers such as polyacetylene, polypyrrole, polythiophene and polyaniline are used as enzyme immobilization carriers, which can effectively improve the sensor conductivity.[78,79] As shown in Figure 11A, Chauhan[80] electropolymerized a layer of polymer (indole-5-carboxylic acid) on a gold electrode surface and then modified the polymer with ZnS. EDC/NHS acted as a crosslinking agent to immobilize the enzyme on the polymer surface. The SEM results showed that the conductive polymer had a rough, uneven surface, (Figure 11B) and that the enzyme was firmly attached to the polymer surface (Figure 11C). The sensor detection limit for

chlorpyrifos was 0.1 pM, and the detection accuracy was 95–100%. Georges[77] synthesized PEDOT:PSS polymer film with good conductivity as an enzyme carrier. Compared with the common conductive polymer medium cobalt phthalocyanine or tetracyanoquinodimethane, PEDOT:PSS has better conductive properties. PEDOT: PSS modified carbon printed electrode. The modified printed electrode had almost the same shape as the unmodified electrode and a resistivity reduced by half. The detection limit of this sensor for chlorpyrifos-oxon was 4 nM, and the error does not exceed 5%. Common non-conductive polymer materials include nylon mesh,[81] Pall Biodyne™,[82] nitrocellulose membrane,[83] pHEMA[84] and PVA-SbQ membrane.[85] Although the non-conductive polymer materials have poor conductivity, they generally have active amino groups and carbonyl groups on their surface, and they can easily fix the enzyme chemically. Researchers have also reported OP detection sensors developed through the covalent coupling of enzymes to immobilize them on nylon meshes surface and through adsorption and crosslinking methods to immobilize OPH on nylon meshes.[86]

Conclusion

The OP sensor is characterized by convenient operation, speed, and easy miniaturization and integration, as well as low preparation cost, which is expected to promote its usage. The detection limit of this sensor is much lower than the minimum pesticide residue standards established by various countries or organizations; however, the sensor stability and repeatability need to be improved, and the differences in test results for different pesticides are also significant. Although researchers use different materials and different structures to fabricate to apply the sensor to actual detection, more accurate signal conversion and signal acquisition technologies need to be developed. In the current research, the use of biosensors to detect OPs still has some limitations, such as the easy loss of enzymes during the detection and the susceptibility of enzyme activity to external influences, resulting in unsatisfactory repeatability of detection results. These factors hinder the large-scale promotion of biosensors. Given the ongoing research on the above problems, biosensors could emerge as worthy alternatives to the conventional chromatography technology. As people gradually prioritize a healthy diet, the research on OPs sensors will also speed up. In addition, patterning and integration maybe also a promising research direction.

Competing interests

The authors declare that there are no competing interests.

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Data availability statement

The date generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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