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REVIEW

Advances in pesticide biosensors: current status, challenges, and future perspectives

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Abstract Public concern over pesticide residues has been increasing dramatically owing to the high toxicity and bioaccumulation effects of pesticides and the serious risks that they pose to the environment and human health. It is therefore crucial to monitor pesticide residues by using various analytical methods and techniques, especially highly sensitive, highly selective, simple, rapid, cost-effective, and portable ones. Biosensor strategies have become research hotspots and ideal candidates for pesticide detection, having such features as high sensitivity, fast response, robustness, low cost and miniaturization, as well as in situ and real-time monitoring. This review covers advances in the design and fabrication of biosensors for pesticide detection since 2005. Special emphasis is placed on the state-of-art selection of receptors, the use of different transduction techniques and fast screening strategies, and the application of various biosensors developed in food and environmental safety. Both advantages and drawbacks of these techniques are then summarized. Finally, challenges, strategies, and perspectives in further developing pesticide biosensors are also discussed.

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Introduction

Pesticides, in general, are chemicals used worldwide in agricultural production to destroy or control weeds, insects, fungi, and other pests [1]. Despite their merits, pesticides are considered to be some of the most dangerous environmental contaminants because of their ability to accumulate and their long-term effects on living organisms. The presence of pesticides in the environment is particularly hazardous, and exposure to these pesticides leads to several health problems that range from asthma attacks, skin rashes, and chronic disorders to neurological diseases [2–5]. In response of their high toxicity and the serious risk that they pose to the environment and human health, intense research efforts have been directed over the years to the development of sensitive and selective schemes for detection of pesticides.

Currently, over 800 active ingredients, which belong to more than 100 substance classes, are present in a wide range of commercial pesticides. Organochlorine, organophosphorus (OP), and organonitrogen pesticides (referring to carbamates, triazines, and their derivatives) are the most important groups. Among them, organochlorine pesticides are more toxic to many organisms and persist in the environment for more than 30 years; therefore, they are beginning to be withdrawn and replaced by compounds that are biodegraded quickly. In place of organochlorine pesticides, OP and organonitrogen pesticides have become the most popular pesticides (insecticides) because of their shorter environmental half-life, relatively lower mammalian toxicity, wide range of applications, and price. The chemical and physical properties of pesticides may differ considerably. This diversity makes the analysis of environmental samples for the presence of pesticides very difficult.

The identification and quantification of pesticides are generally based on gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, or high-performance liquid chromatography–mass spectrometry [6–28]. These methods permit precise and accurate detection and quantification of trace levels of hundreds of these chemicals. However, these conventional methods for monitoring pesticide require time-consuming extraction and cleanup steps, highly trained personnel, expensive equipment, and are not convenient for on-site analysis.

The development of biosensors for pesticides has been an active research area for some years [29–33] in response to the demand for rapid, simple, selective, and low-cost techniques for pesticide detection. Biosensors offer great advantages over conventional analytical techniques, including high specificity for real-time analysis in complex mixtures, high sensitivity, simple operation without the need for extensive sample pretreatment, and low cost.

A literature survey using Thomson Reuters Web of Science reveals that since 1989 more than 1,400 journal articles, book chapters, and patents on pesticide sensors have been published. As illustrated in Fig. 1, the overall number of publications has increased exponentially over the past 20 years, of which 40–50 % are based on enzyme-inhibition mechanisms. Generally, pesticide biosensors can be classified into two major categories: enzyme-based biosensors [30, 31, 34–38] and immunosensors [39–42]. The history and previous achievements of pesticide biosensors before 2005 have been described and reviewed extensively in the literature [34, 37, 38, 41–50]. Because of great progress in the field of pesticide biosensors in the past 5 years (Fig. 1) as well as few recent reviews on them [34, 37, 41], this article briefly covers the mechanisms of using natural enzymes and antibodies, artificial macromolecules, and whole cells as the sensing elements for pesticide biosensors, followed by a survey of new developments in transducers of biosensors for pesticide detection over the last 8 years (some literature prior to 2005 may be included in order to maintain the integrity of the review). The general application of various types of biosensors in food and environmental safety is then introduced. Finally, limitations of each system are discussed and future prospects for developing pesticide biosensors are also proposed.

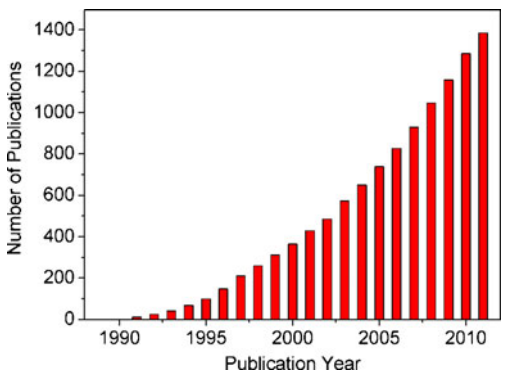

Fig. 1 Number of publications as a function of the year of publication on the research topic of pesticide (bio)sensors from 1989 to 2011 according to Thomson Reuters Web of Science

Fundamentals of pesticide biosensors

Enzyme-inhibition-based biosensor

Since the pioneering work of Guilbault et al. [51] on cholinesterase (ChE) purification, inhibitor analysis, and assay development, biosensors based on the principle of enzyme inhibition have become an active research area. In enzyme-inhibition-based pesticide biosensors, the biological receptors are usually acetylcholinesterase (AChE), butyrylcholinesterase (BChE), or urease. OP and carbamate pesticides selectively inhibit ChEs by blocking the serine in the active site through nucleophilic attack to produce a serine phosphoester (via phosphorylation) [52]. The general principle of the biosensors developed is based on the correlation between the toxicity of pesticides and the decrease of enzyme activity. Therefore, development of these biosensing systems relies on a quantitative measurement of the enzyme activity before and after exposure to a target analyte. Typically, the enzyme-inhibition-based biosensor includes the following steps: determination of initial enzymatic activity, then incubation of a biosensor in a solution that contains pesticides (Eq. 1), and finally measurement of the residual activity (activity after exposure of the biosensor to the pesticide). This activity can be measured by employing different transduction techniques, e.g., amperometry, potentiometry, spectrometry, fluorimetry, and thermometry, for detection of different substrates or products of the enzymatic reaction. For example, in the case of the amperometric method, the assay is based on the electrochemical measurement of thiocholine produced from the following reaction:

$$\text{Acetylthiocholine} + H_2O \xrightarrow{\text{AChE}} \text{Acetic acid} + \text{Thiocholine} \quad (1)$$

The percent inhibition of enzyme activity that results from exposure to pesticides is quantitatively related to the pesticide concentration.

Organophosphorus hydrolase based biosensor

The basis of the mechanism of an organophosphorus hydrolase (OPH)-based biosensor is that OPH is able to hydrolyze a number of OP pesticides, such as paraoxon, parathion, coumaphos, diazinon, chlorpyrifos, and methyl parathion, [53–56], leading to production of organophosphorus acid

and alcohol as a result of the cleavage of the P–O, P–F, P–S, or P–CN bonds (Eq. 2) [57–61].

$$\mathrm{OP} + \mathrm{H_2O} \xrightarrow{\mathrm{OPH}} \mathrm{OP} - \mathrm{acid} + \mathrm{R} - \mathrm{OH}(\mathrm{or}\,\mathrm{R} - \mathrm{SH}) \qquad (2)$$

In many cases, the alcohol produced is chromophoric and/or electroactive. Therefore, the enzyme reaction illustrated in Eq. 2 can be combined with a variety of transduction schemes to construct simple biosensors for direct and rapid determination of OPs [30]. The transducers used include potentiometric transducers [62], optical transducers [63, 64], and amperometric transducers [65, 66]. Because the detection involves the conversion of the substrate to a product, as against inhibition in the case of AChE-based biosensors, such OPH-based biosensors are reusable. Moreover, the immobilization of enzyme is a key step in optimizing the analytical performance of enzyme-based biosensors. Various methods and new materials such as nanomaterials have been employed in immobilizing enzymes to improve stock stability and sensitivity of the enzyme-based biosensors.

Whole-cell biosensors

The first whole-cell biosensor was based on the photosynthetic activity commonly observed in cyanobacteria and microalgae. Approximately 30 % of commercial herbicides inhibit photosynthetic activity or the phosphorylation process [67]. This inhibiting effect can be detected by the Clark-type oxygen electrode, amperometric detection of electron mediators, or optical methods [29, 68–71]. However, these usually have low selectivity, which reduces their attractiveness as potential bioreceptors. Recently, whole-cell sensors based on OPH expressed in periplasm and cytoplasmic membranes have been constructed with *Pseudomonas diminuta* [72], *Flavactenium* sp. [73], and *Sphingomonas* sp. [74–76] that contain the OP-degrading gene (*opd*) as well as recombinant *Moraxella* sp. [77–79], *Pseudomonas putida* [80–84], or *Escherichia coli* with surface-expressed OPH [85–87]. The detection principle is identical to that for the OPH-based biosensor. OPH expressed in whole cells or on the cell surface catalyzes hydrolysis of OPs such as paraoxon, parathion, and methyl parathion to release *p*-nitrophenol [74], which can be detected by electrochemical [74, 77, 85, 87–90] or colorimetric [74] methods. In addition, Chouteau et al. [70] reported a conductometric biosensor using immobilized *Chlorella vulgaris* microalgae as bioreceptors for detection of pesticides and heavy metal ions. The detection relied on the inhibitory effect of OP and carbamates on esterases. The advantages of whole-cell biosensors include low cost, a simple immobilization process, and better stability than enzyme-based sensors. Moreover, because the enzyme is expressed in periplasm and cytoplasmic membranes of cells, whole cells can be immobilized directly onto the transducers for biosensor applications, which do not require the completed immobilization process. Excellent reviews describing the development and application of microbial biosensors for detection of OP pesticides have been published [91–93].

More recently, hybrid biosensors made by co-immobilizing the enzyme and bacteria as bioreceptors have been constructed to improve the selectivity and sensitivity for detection of OPs [94, 95]. Lei et al. [94] reported construction of a hybrid biosensor for determination of OP pesticides with a *p*-nitrophenyl substituent using a purified OPH and *Arthrobacter* sp. J S443 hybrid biorecognition element. The biocatalytic layer was prepared by co-immobilizing the bacteria and AChE on a carbon paste electrode. OPH catalyzed hydrolysis of OP pesticides with a *p*-nitrophenyl substituent such as paraoxon and methyl parathion to release *p*-nitrophenol, which was oxidized by the enzymatic machinery of *Arthrobacter* sp. JS443 to carbon dioxide through the electroactive intermediates 4-nitrocatechol and 1,2,4-benzenetriol. The oxidization current of the intermediates was correlated to the concentration of OPs. The limit of detection (LOD) of the biosensor was as low as 2.8 ppb paraoxon and 5.3 ppb methyl parathion without interference from phenolic compounds, carbamate pesticides, triazine herbicides, and other OP pesticides. A similar hybrid electrochemical biosensor was constructed by using immobilized *Arthrobacter globiformis* and free AChE as a biorecognition system, and a Clark dissolved oxygen electrode was used as the transducer [95]. The LOD for chlorophos was found to be of the order of nanomoles per liter, similar to that achieved with enzyme-inhibition-based sensors for chlorophos quantification. Both these approaches combine the advantages of the bacterial sensors with those of enzyme biosensors: stable response, simple fabrication, and long lifetime at ambient temperature.

Immunosensors

Since AChE is inhibited by not only OP pesticides but also carbamate pesticides and heavy metal ions, most enzyme-inhibition-based biosensors can give a sum parameter of the inhibition efficiency without any qualitative or quantitative information about the individual analytes. Immunobiosensors, which use antibodies to recognize and bind to the desired pesticide, therefore appear to be able to identify a single pesticide or small groups of similar pesticides. Moreover, the antibody–antigen complex can be dissociated by using an appropriate dissociating agent, so theoretically an immunosensor can provide real-time, continuous monitoring of pesticides in a matrix. Immunosensors for pesticides usually operate by competitive inhibition or displacement in which the binding of the free pesticide molecule to the antibody competes with or displaces a tracer molecule. Typically, the analysis consists of two processes: a molecular recognition process and a transduction process. The

transduction process analysis can be achieved by measuring changes in an electrochemical, optical, spectroscopic, or electrical parameter of the receptor caused by the specific binding. The major challenge for immunosensors is the development of appropriate antibodies with desired specificity and high binding ability. Especially, for those pesticides with very low molecular weight or nonrigid structures, it is difficult to develop antibodies. Antibodies have been developed and reported for fewer than 70 pesticides out of over 800 active ingredients, and the corresponding immunosensors are mainly used for detection of organochlorine [96–113] and OP [3, 101, 106, 114–119] pesticides and carbamate insecticides [42, 107, 111, 120–125]. Furthermore, since the response, sensitivity, stability, and reusability of an immunosensor largely depend on immobilization of antibodies onto a transducer or a support matrix, various immobilization strategies including physical or chemical methods have been exploited. Depending on the antibodies and the technique of immobilization, immunosensors may be regenerated a few times by use of different solutions. There are some excellent reviews on immunosensors for pesticide monitoring [39–42].

Artificial-receptor-based biosensors

Sensors based on biological enzymes and antibodies have some intrinsic drawbacks, such as high cost, lack of suitable antibodies, instability, and sometimes too strong binding, which complicates regeneration and prevents reuse. These limitations have generated the need to investigate potential artificial receptors as recognition elements. Among artificial receptors, molecularly imprinted polymers (MIPs), which are artificially synthesized macromolecular materials with a prearranged structure and specific molecular recognition ability for the analytes of interest, have proven their potential as synthetic receptors. In contrast to antibodies and enzymes, MIPs are obtained from relatively simple molecular building blocks using template-directed synthesis, which is considered one of the simplest, most straightforward, cost-effective methods for artificial receptors. Moreover, MIPs have chemical inertness, long-term stability, and insolubility in water and most organic solvents, which make them good candidates in chemical analysis of real samples [126–129]. Recently, the use of MIPs as specific preconcentration elements [130–141] or sensing platforms [53, 133, 142–154] for OPs has been developed. Typically, MIP-based sensors involve two processes:

1. Assembling MIP materials onto the surface of transducers. The polymerizable functional monomers, which bind to the template (target) molecules, are copolymerized onto the surface of transducers via the traditional bulk polymerization method or electropolymerization. After polymerization, the template molecules are removed by extensive washing steps to leave specific recognition sites in the polymer matrix. The functional monomers used for MIP-based pesticide biosensors include acrylic and vinyl monomers [147, 155], methacrylic acid and methacrylate with ethylene glycol dimethacrylate [130, 135, 140, 149, 153, 156], pyrrole [157, 158], acrylic acid [148], 4-vinylpyridine [139, 151, 159], polyethyleneimine [143], *p-tert*-butylcalix[6]-1,4-crown-4 [145], silicates [133, 146], polyamine–thiophenol [152], Co^{2+}–imidazole complexes [144], 2-(trifluoromethyl)acrylic acid [137], polyhydroxyphenol [160], reversible addition–fragmentation transfer polymerization with an *N*-methacryloyl-histidine–Cu^{2+} complex [161], 3-mercaptopropionic acid, and *o*-phenylenediamine [162].
2. Transformation of analyte binding into a measurable signal. Therefore, the efficiency of MIP-based sensors is largely dependent on the selectivity and sensitivity of the MIP materials used to target species.

Unfortunately, although traditional MIP materials exhibit high selectivity, they has some limitations, such as low binding capacity, poor site accessibility, and slow binding kinetics because most imprinted binding sites are embedded in the interior of a highly rigid polymer matrix [163]. Therefore, in order to create more effective recognition sites and improve the accessibility of sites, some groups used nanomaterials as the support materials for preparation of core–shell structural MIPs [160]. A wide range of transducers have been developed in recent years with MIP-based sensors. The first type of frequently used transducers is the optical transducer [164], such as fluorescence assays [147], surface plasmon resonance (SPR) [165], and chemiluminescence [142, 148–151, 166]. Electrochemical transducers are also frequently used in MIP-based biosensors because these sensors are easy to use, rapid, sensitive, and inexpensive [133, 143, 145, 152, 153, 167–170]. The third type of transducer used in combination with MIPs is the mass-based transducer, such as the quartz crystal microbalance (QCM) [154]. Several excellent and comprehensive reviews describing the development of biosensors have been published [171, 172].

Nucleic acid aptamers are an emerging class of synthetic ligands that have great potential as analytical tools for small-molecule detection [173, 174]. Aptamers are artificial single-stranded DNA or RNA sequences (more recently, peptides), and they are thought to resemble chemical antibodies because they interact with their targets with high binding affinity and specificity comparable with the interactions between antibodies and antigens. Additionally, since aptamers have high thermal stability, long shelf-life, and easy chemical synthesis and modification, in addition to their affinity and specificity, these unique features make

them a more effective choice than antibodies. However, aptamers are seldom used as biosensor recognition elements for pesticide detection owing to the complicated process of aptamer selection. Recently, He et al. [175] applied systematic evolution of ligands by exponential enrichment (SELEX) technology to obtain an acetamiprid-specific aptamer with an apparent dissociation constant (K_d) of 1.11 ppm. Although the affinity of the acetamiprid-specific aptamer obtained is lower than that of typical antibodies and the aptamer selection process is very time-consuming (18 rounds of repeated selection), new developments and improvements in the aptamer selection process, rational design of the library, and optimization of the selection conditions could improve the speed of aptamer-based research and result in aptamers highly specific for target pesticides being obtained. Structural studies have revealed that aptamers can frequently undergo significant conformational modifications upon binding to targets [176, 177]. These structural changes could be identified by several transducer strategies, such as mass-responsive or electrochemical or optical biosensors. Therefore, as long as a valid screening method for aptamer generation is available, a simple, portable, and reliable biosensing system for field determination of pesticides in agricultural products and environmental samples should be readily achievable by coupling pesticide-specific aptamers with the sophisticated and elegant transducers.

Transducers of biosensors for pesticide detection

A number of promising transducers are currently being used for pesticide detection. On the basis of the transducing elements, the pesticide biosensors can be categorized as optical, electrochemical, and piezoelectric sensors and rapid screening methods. Here, we present an overview of representative approaches since 2005 according to different detection methods. Table 1 summarizes recent developments in biosensors for pesticide detection.

Optical biosensors

The optical transducer is one of the most convenient and simplest means of chemical detection. Optical biosensors may involve direct detection of the analytes of interest or indirect detection by generating an optical change, such as a change in absorption or fluorescence intensity/color. The advantages of optical biosensors are their analytical speed, the resistance of the signals to electrical or magnetic interference, and the potential for higher information content (multiple signals based on varying peak positions are available). The major disadvantage is the high cost of some instruments.

Fiber-optic biosensors

Fiber-optic biosensors provide some advantages over other transducers, such as no need for direct electrical connection, ease of miniaturization, possibility of remote sensing, and in situ monitoring [178]. In addition, fiber-optic biosensors minimize many of the undesired interactions between the fluorescent probe and samples because the biorecognition elements are entrapped within a protective polymer matrix. The matrix allows substrates to diffuse easily and bind with the biorecognition elements, but prevents release of the biorecognition elements into samples. Recently, fiber-optic enzyme biosensors for detection and determination of pesticides have been reported [63, 179–185]. For example, a fiber-optic biosensor based on sol-gel immobilized ChE might detect dichlorvos at a concentration as low as 5.2 ppb [179]. Andreou and Clonis [186] reported a fiber-optic biosensor based on the enzyme glutathione *S*-transferase I for detection and determination of atrazine. The LOD of the sensor was 0.18 ppm and the concentration range was 0.54–27.0 ppm. Choi et al. [187] developed a fiber-optic biosensor by co-immobilizing AChE and glutathione *S*-transferase in a sol-gel film. The proposed biosensor successfully detected captan and OP compounds at a concentration from 0 to 2 ppm.

A fiber-optic-based microbial biosensor was designed for determination of methyl parathion. The proposed biosensor contained a disposable microbial membrane, in which *Flavobacterium* sp. MTCC 2495 was entrapped on a glass fiber filter (Fig. 2) [74]. The detection is based on hydrolysis of methyl parathion by OPH in *Flavobacterium* sp., resulting into the chromophoric product *p*-nitrophenol being formed. The disposable biocomponent was cost-effective and needed only 75 μL of sample. The LOD of the proposed microbial sensor was 78.96 ppb and the linear range was 1.05–21.06 ppm.

This technique was also applied for development of fiber-optic immunosensors. For instance, Long et al. [188] developed a new portable miniaturized evanescent wave all-fiber immunosensor for detection of 2,4-D. The calibration curve obtained for 2,4-D had an LOD of 0.07 ppb, and the quantitative detection range was 0.22–69.5 ppb. The regeneration of the sensor surface allowed more than 100 assay cycles to be performed without significant loss of reactivity.

A fiber-optic biosensor based on MIPs was obtained by direct polymerization of a vinyl benzoate ligating molecule [189, 190] on a fiber-optic probe. A luminescent lanthanide (europium) was incorporated into the polymer to act as a signal transducer. This type of sensor had an LOD of parts per trillion or lower.

Fluorescent biosensors

Fluorescent biosensors offer significant advantages over other conventional methods for detection of pesticides. The major

Table 1 Recent developments in biosensors for pesticide detection

Transduction system	Pesticides	LOD	Year/reference
Optical biosensors			
Fiber-optic methods			
ChE-based fiber-optic biosensor	Dichlorvos	5.2 ppb	2002 [179]
OPH-based fiber-optic biosensor	Paraoxon	7 ppt	2005 [341]
Fiber-optic immunosensor	2,4-D	70 ppt	2008 [188]
OPH-based fiber-optic fluorescence biosensor	Paraoxon	275.2 ppb	2006 [198]
Whole-cell fiber-optic biosensor	Methyl parathion	78.96 ppb	2006 [74]
MIP-based fiber-optic biosensor	Pyrethroids	3.6 ppb	2010 [146]
Fluorescence			
ChE-based fluorescent biosensor	Paraoxon	0.5 ppt	2011 [205]
OPH-based fluorescent biosensor	Paraoxon	1.38 ppb	2006 [203]
Fluorescent immunosensor	Trichloropyrindinol	1.0 ppb	2010 [96]
MIP-based fluorescent biosensor	Diazinon	50 ppb	2011 [147]
SPR			
Immuno SPR biosensor	Paraoxon	50 ppt	2007 [99]
ChE-based SPR biosensor	Paraoxon	1 ppb	2006 [244]
MIP-based SPR biosensor	Acephate	7.86×10^{-3} ppt	2011 [165]
Surface-enhanced Raman scattering	Paraquat	0.51 ppb	2010 [248]
Electrochemical methods			
ChE-based amperometric biosensor	Paraoxon	0.11 ppt	2006 [258]
OPH-based amperometric biosensor	Parathion	1 ppb	2000 [288]
Amperometric immunosensor	Paraoxon	1.38 ppb	2010 [269]
ChE-based potentiometric biosensor	Dichlorvos	0.05 ppb	2009 [280]
Conductimetric immunosensor	Paraoxon	0.5 ppb	2008 [342]
Whole-cell amperometric biosensor	Methyl parathion	263.21 ppb	2005 [80]
FET	Atrazine	0.2 ppb	2003 [343]
MIP-based potentiometric biosensor	Triazophos	29.14 ppb	2012 [152]
MIP-based amperometric biosensor	Paraothion	3 ppb	2009 [143]
Piezoelectric sensors			
QCM	EPN	5 ppb	2007 [300]
Microcantilever biosensor	Paraoxon	27.5 ppb	2007 [344]
MIP-based QCM sensor	Atrazine	20 ppb	2011 [156]
Rapid screening			
Immunostrip	Metolachlor	0.16 ppb	2009 [308]
ChE-based colorimetric strip	Paraoxon	0.275 ppb	2009 [321]

ChE cholinesterase, *FET* field-effect transistor, *LOD* limit of detection, *MIP* molecularly imprinted polymer, *OPH* organophosphorus hydrolase, *QCM* quartz crystal microbalance, *SPR* surface plasmon resonance

advantages of fluorescence are its high single-molecule sensitivity, and in most cases, an instantaneous response. The concentrations of analytes that can be measured by fluorescence methods are 10^6 times smaller than those that can be measured by absorbance techniques [191]. To date, a number of sensitive fluorescent biosensors have been developed for pesticide detection based on monitoring the change in fluorescence intensity generated in enzymatic reaction or antibody–antigen specific binding. The change in fluorescence intensity is proportional to the pesticide concentration. An excellent review on fluorescent sensors for detection of OP compounds is available [192]. So, herein we focus our discussion on recent developments of fluorescent pesticide biosensors.

Enzyme-based fluorescent sensors for detection of pesticides can be categorized into two general classes on the basis of the enzyme employed: (1) AChE/BChE and (2) OPH. Hydrolysis of acetylcholine by AChE produces a proton, resulting in an increase of the acidity. OPH catalytically hydrolyses OP pesticides to produce two protons in each hydrolytic turnover. Therefore, most AChE-based and OPH-based biosensors rely on visualizing pH changes through pH-responsive fluorophores. Such pH-sensitive fluorescent probes are mainly different types of organic dyes, including pyranine [193], fluorescein isothiocyanate [64, 194, 195], 2-butyl-6-(4-methylpiperazin-1-yl)benzo[*de*]isoquinolin-1,3-dione [196], carboxyseminaphthofluorescein [197], carboxynaphthofluorescein [198], and

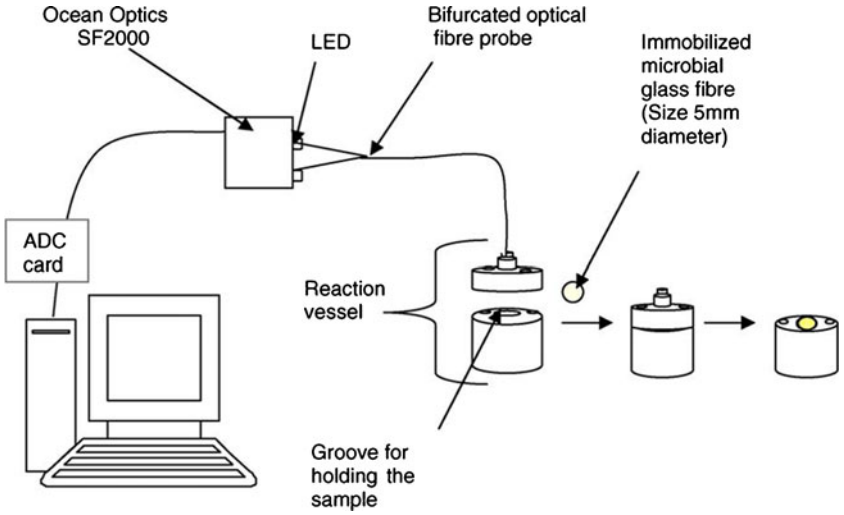

Fig. 2 The operating system composed of a fiber-optic biosensor and reaction vessel. *ADC* analog-to-digital converter. (From [74]. Copyright 2006 Elsevier)

so on [199–202]. Vamvakaki and Chaniotakis [193] used this approach by immobilizing AChE and the pH-sensitive fluorescent indicator pyranine in liposomes. In the absence of pesticides, AChE was able to hydrolyze acetylcholine to choline and acetic acid, resulting in a reduction of the solution pH. This pH decrease, in turn, led to quenching of pyranine fluorescence. This biosensor was capable of detecting dichlorvos as low as 44.2 ppt and paraoxon as low as 183.4 ppt. Orbulescu et al. [203] demonstrated that covalent immobilization of fluorescently labeled OPH on a silanized quartz substrate resulted in an increase of enzyme stability, and an LOD in the nanomolar concentration regime could be realized. OPH-based fluorescent sensors have been further used in portable electronic devices. Viveros et al. [198] reported an OPH biosensor in which the fluorescent dye carboxynaphthofluorescein was covalently bound to the enzyme. The complex was reported to operate well in combination with a fiber-optic biosensor assay. The fluorescence intensity of carboxynaphthofluorescein decreased with decreasing pH, and through correlation of the fluorescence intensity with the concentration of OP, an LOD of 4.76 ppt was achieved.

Other fluorescent probes have also been developed. Typical inorganic examples include gold nanoparticles (AuNPs) [204], semiconductor fluorescent nanoparticles [205–214], and Eu^{3+} ions [215–217]. For example, Simonian et al. [204] reported the use of OPH as the receptor for OP analytes. In this system a phosphate derivative of a fluorophore was weakly bound to the enzyme, which in turn was covalently attached to AuNPs (Fig. 3). If the AuNP attached to the OPH was at a certain distance from the fluorophore (typically between 10 nm and 40 nm), enhancement of fluorescence was observed. If the substrate was present, the analyte displaced the fluorophore and tightly bound to the enzyme active site. As the fluorophore was removed from AuNPs, its fluorescence intensity decreased owing to loss of the enhancement effect of AuNPs. Semiconductor-nanoparticle-based fluorescent enzyme biosensors [205–214] for pesticides are also documented in the literature. Leblanc's group [210] reported a novel biosensor for detection of paraoxon based on CdSe–ZnS core–shell nanoparticles and an OPH bioconjugate. The biosensing mechanism was based on the conformational change in the enzyme influencing the degree of surface passivation of semiconductor nanoparticles. The LOD for paraoxon using the OPH/nanoparticle bioconjugate was about 2.75 ppt. Recently, our group [205, 214] developed two systems for detecting OP pesticides via incorporating fluorescent CdTe nanoparticles, choline oxidase (ChOx), and AChE into nanostructured films by using a layer-by-layer assembly technique (Fig. 4). The proposed biosensors were highly sensitive to OP pesticides on the basis of the change in fluorescence of the nanostructured film, and the LODs were as low as picomolar levels.

Beside enzyme-based biosensors, a number of biosensors have been developed based on fluorescence immunoassays [96, 110, 116, 118, 218–220]. Additional information can be found in a review by Jiang et al. [41].

Fluorescence-based MIP sensors have also been applied for detection of OP pesticides and insecticides. Jenkins et al. [215, 216] developed a supramolecular fluorescent sensor for detection of OP compounds by employing an MIP, [Eu $(dvmb)_3(pmp)(NO_3)_2$] (dvmb is 3,5-methyl divinylbenzoate; pmp is pinacolyl methylphosphonate), in combination with Eu^{3+} ions. The sensor showed remarkable sensitivity. The LOD was 7 ppt for a portable system, with a linear range from 10 ppt to 10 ppm, and showed no false positives for chemically similar OP compounds, such as dichlorvos [216]. Southard et al. [221] used complexes of a dithiobenzoate-substituted tris(β-diketonate) europium (III) complex and the lanthanide europium(III) as the polymerization substrate and as a luminescent binding site for pinacolyl methylphosphanate. The detection was based on changes that occur in the spectrum of a europium(III) tris

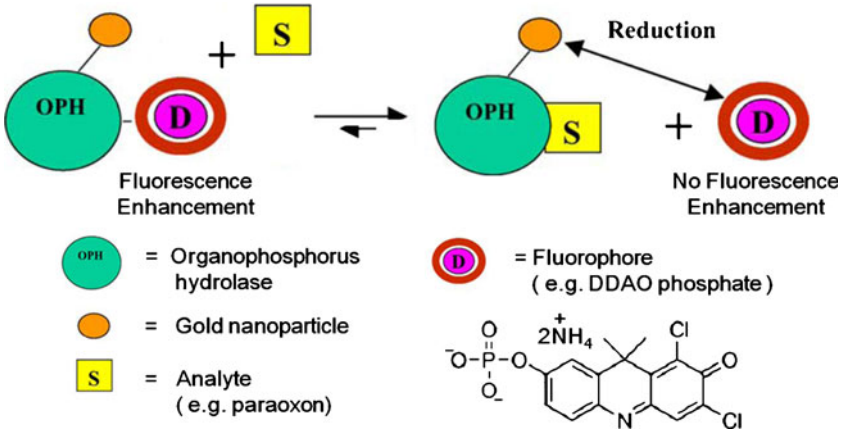

Fig. 3 Analyte displacement of a fluorophore—diammonium 9 *H*-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl phosphate (*DDAO phosphate*)—from an organophosphorus hydrolase (*OPH*)–gold complex, leading to a reduction in fluorescence. (Reproduced with permission from [204]. Copyright 2005 Elsevier)

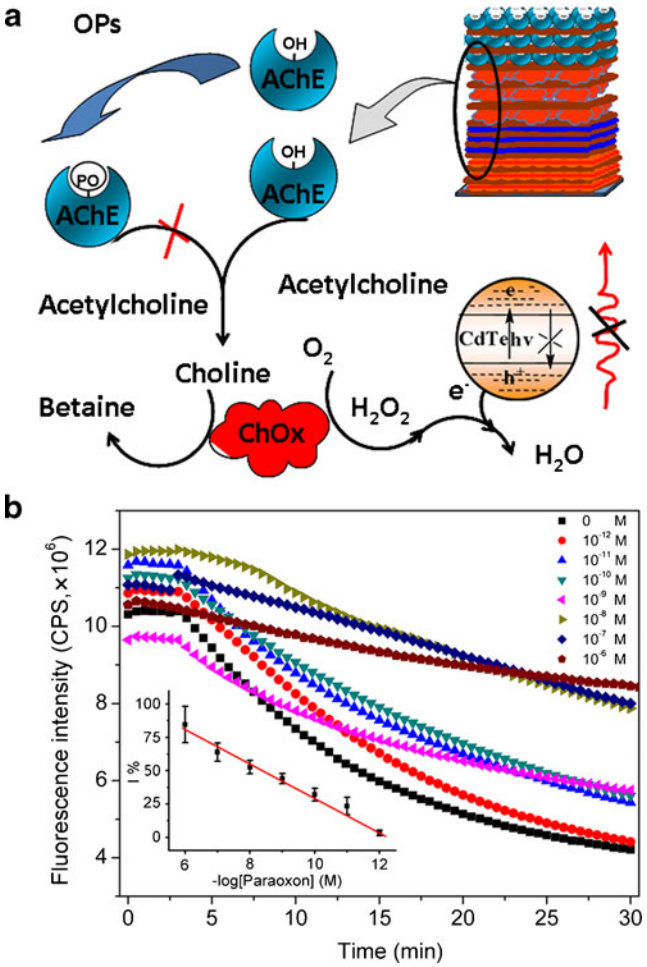

Fig. 4 **a** Fluorescent biosensor based on multilayers of CdTe nanoparticles, choline oxidase (*ChOx*), and acetylcholinesterase (*AChE*). **b** Fluorescence changes at 592 nm upon the interaction of the biosensor with various concentrations of paraoxon. The *inset* shows inhibition curves of the proposed biosensor for different concentrations of paraoxon. Linear response ranges were obtained from 2.75×10^{-4} to 2.75 ppt. *CPS* counts per second, *OPs* organophosphates. (Reproduced with permission from [205]. Copyright 2012 Royal Society of Chemistry)

chelate upon pinacolyl methylphosphanate binding. A combination of good sensitivity and very high selectivity was obtained with this method. The sensitivity of fluorescence-based MIP sensors can be improved by using nanomaterials as the support materials for the MIPs [146, 147, 222]. For example, Li et al. [146] developed an MIP-based fluorescence nanosensor by anchoring the MIP layer on the surface CdSe quantum dots (QDs) embedded in silica nanospheres via a surface molecular imprinting process. The synthesized CdSe–SiO_2–MIP showed higher photostability, and allowed highly selective and sensitive determination of lambda-cyhalothrin. Under optimal conditions, the relative fluorescence intensity of CdSe–SiO_2–MIP decreased linearly with increasing lambda-cyhalothrin concentration in the range from 0.45 to 449.9 ppm, with an LOD of 3.6 ppb. Zhao et al. [147] prepared QD-based MIP composite nanospheres. On the basis of the novel fluorescence quenching relying on energy transfer from emission of QDs being absorbed by diazinon in water, the QD-based MIP nanospheres were successfully applied to selective and sensitive detection of diazinon in water. The LOD was 50 ppb, and the linear range was 50–600 ppb.

Surface plasmon resonance biosensor

SPR is an optical technique that reports changes in refractive index occurring on noble metal thin films, e.g., gold films, as a result of chemical events [223]. SPR makes possible real-time, label-free detection of pesticides owing to its high sensitivity and reliable procedure. Immunosensor systems based on SPR detection have been used for various types of pesticides, such as atrazine [224, 225], 2,4-D [226–228], chlorpyrifos [229, 230], carbaryl [231], DDT, i.e., [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] [100], isoproturon [232], and other pesticides [98, 233–241]. A competitive immunoassay based on SPR for detection of 2,4-D was reported [242]. The assay was based on regeneration of the chip surface by the reversible interaction between a monosaccharide (D-glucose) and a lectin (concanavalin A). The

interaction between anti-2,4-D antibody and the surface-bound concanavalin A–2,4-D conjugate was monitored by SPR and the response was used to quantify 2,4-D. The dynamic range of the curve was 3–100 ppb. Multianalyte SPR immunoassays for simultaneous detection of pesticides were reported by Mauriz et al. [98] and Yang and Kang [243]. These portable immunosensors based on SPR technology provided highly sensitive detection of pesticide analytes at nanogram per liter levels. The assay reproducibility was proved through the repeated use of the same sensor surface for more than 200 assay cycles.

Coupled with SPR, AChE immobilized on the sensor surface can be used to detect and screen pesticides [244–247]. Lin et al. [244] applied the localized SPR of AuNPs covalently coupled with AChE to create a biosensor for detecting paraoxon in the range from 1 to 100 ppb.

Because enzymes and antibodies are costly and not very stable, artificial receptors such as MIPs were anchored on a gold surface to fabricate SPR sensors for ultrasensitive detection of acephate [165]. The LODs were 0.02 ppt for an apple sample and 0.008 ppt for a cole sample. The imprinted ultrathin film had impressive selectivity. The selectivity efficiencies for acephate and other structurally related analogues were 1.0 and 0.11–0.37, respectively.

Surface-enhanced Raman scattering

Recently, the surface-enhanced Raman scattering (SERS) detection technique using metal nanomaterials as the substrates has been considered as a promising alternative for highly sensitive detection of pesticides [248–255]. Liron et al. [250] developed a sensitive SERS assay for detection of paraoxon based on an AChE-inhibition mechanism. The principle of operation of this assay was based on adsorption of the enzyme-reaction product, thiocholine, on silver nanoparticles to give a specific SERS spectrum (Fig. 5). Variation in the SERS signals was directly correlated with the concentration of the thiocholine produced. The method allowed detection of paraoxon down to 5.0 ppb with a response time of 5 min. Gao et al. [248] reported a rapid and highly sensitive trace analysis of paraquat in water using a SERS-based microdroplet sensor. The LOD for paraquat in water was estimated to be about 0.37 ppb.

Electrochemical biosensors

Electrochemical transducers are the most frequently used transducers in biosensors measuring pesticides in water and foods, because these sensors are easy to use, rapid, sensitive, of low cost (especially in the case of screen-printed electrodes), and well adapted to miniaturization for in situ monitoring. The high reproducibility of these single-use electrodes eliminates the requirement of repeated calibration.

A major percentage of electrochemical biosensors are based on inhibition of the enzyme AChE by pesticides. The main approach used to measure this inhibition is based on the amperometric detection of thiocholine, which is the enzymatic reaction product of acetylthiocholine and is oxidized at constant potential at the electrochemical transducer. The electrochemical methods based on commercial AChE have two shortcomings when used in practical applications: the high LOD and the lack of selectivity. To decrease the LOD, different immobilization approaches and new materials such as AuNPs [256], QDs [257], carbon nanotubes [258–266], graphene [267, 268], and magnetic nanomaterials [269] have been proposed. For example, Liu and Lin [258] incorporated AChE and carbon nanotubes into multilayers by a layer-by-layer assembly technique, as shown in Fig. 6a. The electrocatalytic activity of carbon nanotubes led to greatly improved electrochemical detection of the enzymatically generated

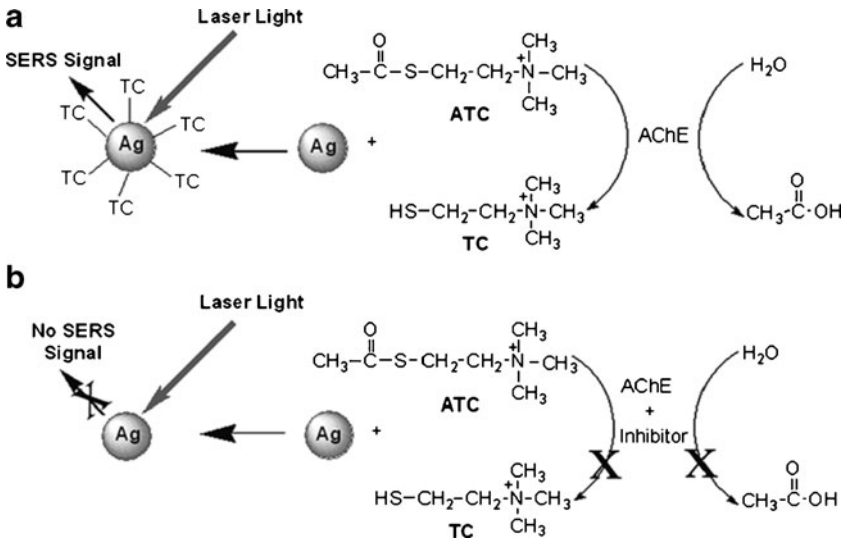

Fig. 5 Detection of AChE inhibitors. Acetylthiocholine (*ATC*) is hydrolyzed by AChE to produce thiocholine (*TC*) and acetic acid, and the thiocholine produced is adsorbed on silver nanoparticles to give a characteristic surface-enhanced Raman scattering (*SERS*) signal (**a**). In the presence of an AChE inhibitor, AChE activity is blocked, no thiocholine is produced, and no SERS signal is produced (**b**). (Reproduced with permission from [250]. Copyright 2011 Elsevier)

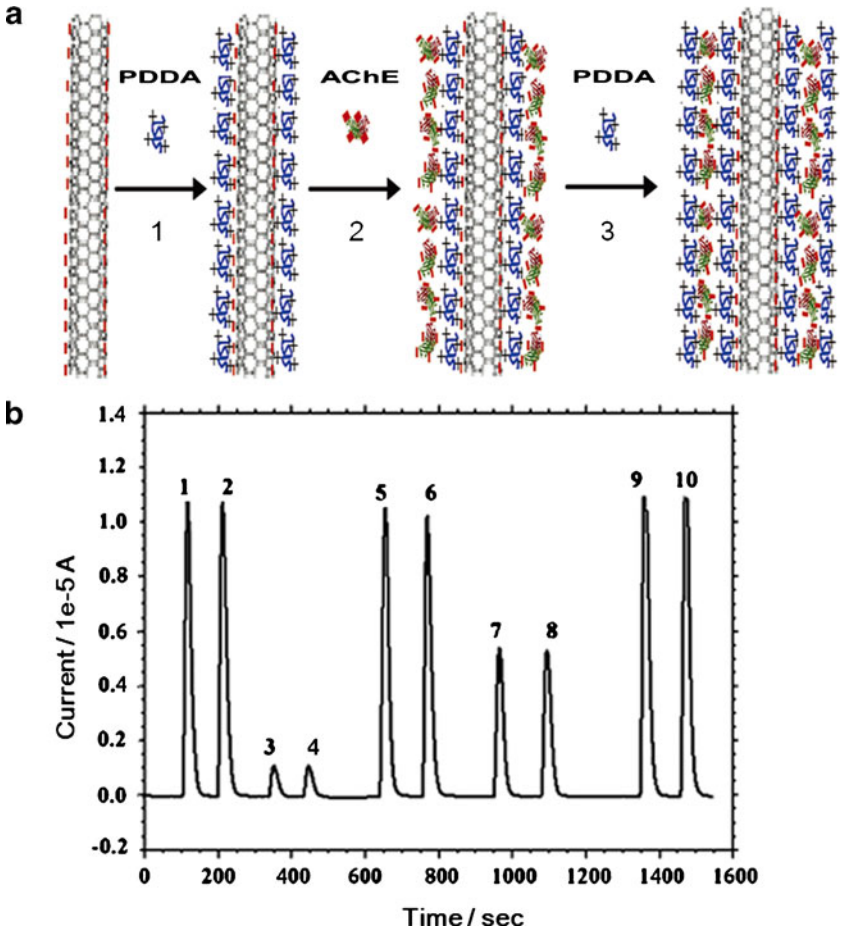

Fig. 6 **a** Layer-by-layer electrostatic self-assembly of AChE on carbon nanotubes (CNTs): (1) assembling positively charged poly(diallyldimethylammonium chloride) (*PDDA*) on negatively charged CNTs; (2) assembling negatively charged AChE; (3) assembling the second PDDA layer. **b** Typical amperometric responses of the PDDA/AChE/PDDA/CNT/glassy carbon electrode biosensor during the flow injection analysis of paraoxon. Signals *1* and *2*, initial enzyme activity; signals *3* and *4*, enzyme activity after incubation for 6 min with 10 μL of 1 $\times 10^{-8}$ M paraoxon; signals *5* and *6*, enzyme activity after regeneration with 1 mM pyridine 2-aldoxime methiodide and 10 mM acetylthiocholine; signals *7* and *8*, enzyme activity after incubation for 6 min with 10 μL of 1×10^{-10} M paraoxon; signals *9* and *10*, enzyme activity after regeneration with 1 mM pralidoxime and 10 mM acetylthiocholine. The current versus time recording was paused during inhibition and regeneration. Flow rate 0.25 mL·min^{-1} working potential 150 mV. (Reproduced with permission from [258]. Copyright 2006 American Chemical Society)

thiocholine product, including a low oxidation overvoltage (+150 mV), higher sensitivity, and higher stability. The biosensor integrated into a flow injection system was used to monitor OP pesticides and nerve agents, such as paraoxon. Under optimal conditions, the biosensor was used to measure as little as 0.11 ppt paraoxon with a 6-min inhibition time (Fig. 6b). The biosensor had excellent operational lifetime stability with no decrease in the activity of enzymes for more than 20 repeated measurements over a 1-week period.

Moreover, developments in biosensor technology using disposable screen-printed carbon electrodes have provided cost-effective and reliable arrays that are suitable for rapid electrochemical assessment of pesticides in situ [270, 271]. Recently, Crew et al. [272] fabricated an electrochemical biosensor array based on screen-printing technology for the rapid identification and quantification of specific OP residues in environmental and food samples (as shown in Fig. 7). The total analysis time for the complete assay was less than 6 min. The array was used to generate calibration data with six OPs in the concentration range from 10^{-5} to 10^{-9} M to train a neural network. Some examples of electrochemical biosensors for determination of residual pesticides are presented in Table 2.

Although amperometry has been the preferred option in most applications, potentiometry [273, 274] and conductimetry have also been employed. The potentiometry methods used a pH-sensitive transducer such as a pH electrode, ion-selective field-effect transistors [275, 276], electrodes modified with polymers [277–279], and a polymeric membrane ionic-selective electrode [280] to measure the variation in pH induced by release of acetic acid during the enzymatic reaction.

In addition to AChE-based electrochemical sensors, OPH-based electrochemical sensors are used for determination of OP pesticides. OPH catalyzes hydrolysis of OP pesticides a

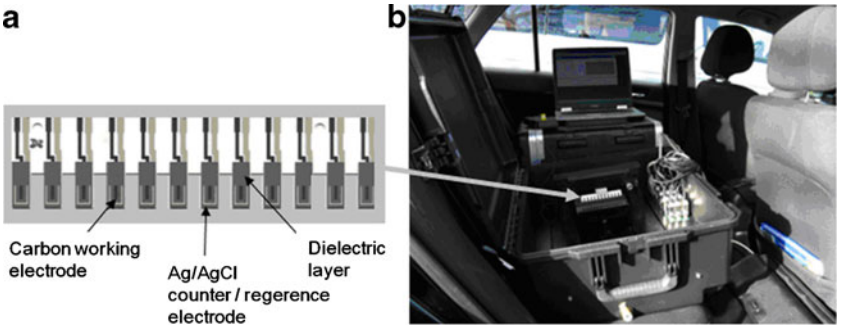

Fig. 7 Electrode array comprising 12 screen-printed carbon electrodes modified with cobalt phthalocyanine and an Ag/AgCl counter/reference electrode printed on an alumina substrate (*a*) and an array in the prototype biosensor system operating in the field powered from a car battery via the lighter socket (*b*). (Reproduced with permission from [272]. Copyright 2011 Elsevier)

with *p*-nitrophenyl substituent and generates *p*-nitrophenol, which can then be detected amperometrically [281, 282] or potentiometrically [283]. Cells expressing OPH in whole cells or on the cell surface were integrated with an amperometric or potentiometric transducer (pH electrode) to construct bacterial electrochemical sensors for OP compounds such as paraoxon, methyl parathion, and diazinon [74, 77, 86].

A significant number of articles on MIP-based electrochemical sensors have also been published [133, 143, 145, 152, 153, 158, 160, 162, 170, 190, 284]. Especially, amperometric detection has recently been used frequently because of its high selectivity. Prasad et al. [285] prepared MIP particles by traditional bulk polymerization, whereas D'Agostino et al. [153] produced MIP membranes by mixing methacrylic acid, ethylene glycol dimethacrylate, atrazine and 2,2′-azobis(isobutyronitrile). Xie et al. [152] reported a surface molecular self-assembly strategy for molecular imprinting in electropolymerized polyaminothiophenol membranes at the surface of an AuNP-modified glassy carbon electrode for electrochemical detection of the pesticide chlorpyrifos. The *p*-aminothiophenol monomer was first assembled on AuNPs at the glassy carbon electrode via Au−S bonds, and subsequently, the chlorpyrifos template was further assembled onto the monolayer of *p*-aminothiophenol through the hydrogen-bonding interaction between the amino group and chlorpyrifos. The MIP membrane was then obtained by the electropolymerization of the assembled electrode in a mixing solution containing additional *p*-aminothiophenol and chlorpyrifos templates. During electropolymerization, the chlorpyrifos was spontaneously imprinted into the electropolymerized polyaminothiophenol membrane/AuNP film. The number of imprinted sites on the electropolymerized polyaminothiophenol membrane/AuNP film was significantly increased owing to the additional replenishment of chlorpyrifos templates. The LOD for chlorpyrifos was about two orders of magnitude lower than that obtained with the imprinted chlorpyrifos/gold sensor. MIP sensors based on potentiometry and conductometry were also developed. Delaney et al. [168] developed a sensor by preparation of photografted molecularly imprinted polymerization on the surface of gold electrodes. The sensor was used to measure changes in the dielectric constant of the insulating layer for the herbicide desmetryn.

Finally, in the literature, there are also reports of other types of electrochemical biosensors based on organophosphorus acid anhydrolase [286], the bienzymatic AChE (BChE)–ChOx system [287], parathion hydrolase [288], methyl parathion degrading enzyme [218], tyrosinase [289], a bacteria–AChE system [77, 290], and antibodies [291–293].

Other transducers

Other detection techniques have been used in order to avoid some limitations found when analyzing real samples, such as interferences from electroactive species in electrochemical sensors and spectral overlapping from other compounds in optical sensors. Among them, photothermal techniques [294], Fourier transform infrared spectroscopy [295], and a piezoelectric biosensor were used for determination of pesticides.

Thanks to their simplicity, low cost, and real-time response, piezoelectric biosensors can be used to measure pesticides [102, 296, 297]. Makower et al. [298] introduced a piezoelectric biosensor for sensitive detection of paraoxon. The ChE inhibitor was immobilized on the sensing surface via a chelate complex as the recognition element. First, the conjugate of *N*-mercaptoundecanoic acid (MUA) with *Nα*-bis(carboxymethyl)-L-lysine (NTA-Lys) was preadsorbed on the surface of the gold electrode of the piezoelectric sensor to form a self-assembled monolayer. Then, a paraoxon–spacer–hexahistidine conjugate was linked to the MUA–NTA-Lys layer via the chelate complex with Ni^{2+}. The paraoxon-modified surface was then applied for binding of human BChE. In the presence of diisopropylfluorophosphate, binding of BChE to the surface-bound paraoxon was decreased. In this way, a competitive affinity assay for diisopropylfluorophosphate in water was achieved with an LOD of 1.84 ppb. Regeneration of the sensing surface was achieved by destroying the chelate complex with EDTA, thus washing out the BChE-inhibitor

Table 2 Some examples of electrochemical biosensors for determination of pesticides

Pesticides	Bioreceptor	Detector	LOD	Detection time	Comments	Year/reference
Dimethyl 2,2-dichlorovinyl phosphate	AChE (E. eel)	Amperometric	1 ppb	Incubation time 3 min	A disposable SPE modified with a nylon membrane containing AChE, combined with a miniaturized flow cell system, allowed fast measurements of pesticides in the environment on the spot	2000 [329]
Parathion	Parathion hydrolase (*Pseudomonas* sp.)	Amperometric	1 ppb	1 min	The combination of parathion hydrolase modified carbon SPE with a microflow injection system and pulsed techniques allowed selective, highly sensitive, and continuous monitoring of parathion	2000 [288]
Chlorpyrifos-ethyl oxon	AChE (D. m)	Amperometric	0.1 ppb	Incubation time 20 min	The histidine-tagged AChE was immobilized on an SPE by a metal chelate, which allowed control of the orientation of the enzyme	2001 [217]
Chlorpyrifos-ethyl oxon	AChE (D. m)	Amperometric	2 ppt	Incubation time 20 min	Three immobilization methods were used for the fabrication of a reproducible and cheap SPE biosensor, including entrapment in a PVA–SbQ photopolymer, a sol-gel matrix, and immobilization by metal–chelate affinity	2002 [345]
Atrazine	Antibody	FET	0.2 ppb	20–25 min	The using of protein A–polyanion conjugates allowed antigens without additional treatment of specific antisera	2003 [343]
Paraoxon	AChE (E. eel) and ChOx (*Alcaligenes* sp.)	Amperometric	27.5 ppt	Incubation time 30 min	A ferro phthalocyanine chemically modified carbon paste electrode coupled with bienzyme in a dialysis membrane operated at a low applied potential (0.35 V) exhibited good stability (several months in a dry state at 4 °C).	2003 [287]
Atrazine	Tyrosinase	Conductometric	1 ppb	30 min	The conductometric tyrosinase biosensor showed a better LOD than that obtained using an amperometric tyrosinase biosensor. It did not need a reference electrode and can be driven at low voltage	2004 [289]
Dichlorvos	AChE (D. m)	Amperometric	22.1 ppt		The immobilization of AChE onto a polyethyleneimine-modified SPE increased storage and operational stability, but resulted in sensitivity loss	2004 [346]
Pirimiphos methyl	AChE (D. m) mutant	Amperometric	1 ppt	Incubation time 30 min	Engineering AChE B led to an increase in sensitivity and extraordinary high storage stability	2005 [33]
Paraoxon	AChE (E. eel)	Amperometric	0.145 ppb	Incubation time 30 min	A low-cost disposable biosensor was developed on the basis of a thick film strip electrode modified with AChE–functionalized acid purified CNTs; CNTs improved the electrocatalytic activity toward thiocholine and functionalized the immobilization matrix for the enzyme	2005 [347]
Omethoate	Wild-type AChE (D. m) and mutant	Amperometric	21.3 ppb	48 min	The use of enzymes from different sources improved the LOD and allowed discrimination of the interferences from mercury and hypochlorite	2005 [348]

Table 2 (continued)

Pesticides	Bioreceptor	Detector	LOD	Detection time	Comments	Year/reference
Paraoxon	OPH	Amperometric	41.3 ppb	10 min	The OPH/CNT-coated SPE showed higher sensitivity and stability owing to the accelerated oxidation of hydrogen peroxide at the CNT transducer	2005 [349]
Paraoxon	AChE	Amperometric	0.11 ppt	Incubation time 6 min	The LBL nanostructures on the CNT surface provided a favorable microenvironment to maintain the bioactivity of AChE and the electrocatalytic activity of the CNTs lowered the oxidation overvoltage. The biosensor integrated with a flow injection system was an ideal tool for online monitoring of pesticides	2006 [258]
Paraoxon	OPH and engineered *Moraxella* sp.	Oxygen electrode	27.5 ppb	5 min	Combining engineered *Moraxella* sp. and OPH with a Clark dissolved oxygen electrode allowed sensitive, selective, rapid, and direct determination of *p*-nitrophenyl-substituted OP compounds	2006 [77]
Atrazine	MIP of MAA/EGDMA	Potentiometric	4.31 ppm	Response time less than 10 s	The response time was less than 10 s and the sensor could be used for more than 2 months without any divergence, but the LOD was high	2006 [153]
Paraoxon	OPH	Conductance	13.8 ppm	Real time	The biosensor based on OPH immobilized on a SWCNT electrode could serve as a simple, reusable, and low-cost biosensing platform	2007 [350]
Triazophos	AChE (E. eel)	Amperometric	1.57 ppb	Incubation time 12 min	AChE was immobilized on silica sol-gel film assembling MWCNTs, in which the sol-gel matrix provided a biocompatible microenvironment and efficiently prevented leakage of the enzyme and MWCNTs promoted electron transfer reactions	2007 [351]
Monocrotophos	AChE (E. eel)	Amperometric	0.6 ppb	Incubation time: 10 min	Immobilization of AChE on AuNPs embedded in sol-gel film provided a biocompatible microenvironment	2007 [352]
Malathion	AChE (E. eel)	Amperometric	0.03 ppb	Incubation time 10 min	AChE immobilized on electrodeposited chitosan film mediated the generation and growth of AuNPs, which as a mediator improved the electron transfer between species in solution and the electrode	2007 [353]
Monocrotophos	AChE (E. eel)	Amperometric	0.3 ppb	Incubation time 8 min	Immobilization of AChE on a CdTe QD–AuNP composite modified chitosan microsphere interface favored the interface enzymatic hydrolysis reaction to form an electroactive substance and prevented enzyme molecules from leaking	2008 [257]
Dichlorvos	AChE	Amperometric	8 ppt	Incubation time 10 min	The AuNP-coated silicon nanowires enhanced the enzymatic activity and facilitated electron communication, thus significantly enhancing the sensor response	2008 [354]
Paraoxon	AChE (he)	Amperometric	0.55 ppt	20 min	Owing to the electrocatalytic properties of CNTs, the CNT-modified SPE integrated with a microflow injection system shows high sensitivity	2008 [259]

Table 2 (continued)

Pesticides	Bioreceptor	Detector	LOD	Detection time	Comments	Year/reference
DNOC	DNOC-imprinted copolymer of aniline and phenylenediamine	Amperometric	39.83 ppb		The microsensor based DNOC-imprinted electrocopolymerized polymeric film was able to differentiate between DNOC and other closely related compounds	2008 [169]
Dichlorvos	AChE	Potentiometric	0.05 ppb	5 min	Enzyme was immobilized on a carboxylated PVC matrix at the outer layer of the ISE membrane. With the reactivation procedure, such a biosensor could be repeatedly used for at least 10 cycles	2009 [280]
Chlorophos	*Arthrobacter globiformis* and AChE (be)	Potentiometric	0.26 ppb	Response time 200 s	The proposed biosensor combined the advantages of bacterial sensors with the sensitivity of inhibition-based AChE ones. The LOD for chlorophos was similar to thatachieved with enzyme-inhibition-based sensors	2009 [95]
Methyl parathion	AChE (E. eel)	Amperometric	1 ppb	Incubation time 10 min	The immobilization of AChE in a calcium carbonate–chitosan based network-like structure provided a favorable and biocompatible microenvironment	2009 [290]
Methyl parathion	AChE (E. eel)	Amperometric	2 ppb	Incubation time 10 min	AChE was immobilized onto an AuNP–polypyrrole nanowire network-like nanocomposite, which not only provided a biocompatible microenvironment to maintain the bioactivity of AChE, but also exhibited a strong synergetic effect and thereby improved the sensing properties	2009 [256]
Atrazine	PAAT-*co*-EDOT	Amperometric	21.57 ppb	Response time 10 s	The sensor based on atrazine-imprinted conducting polymer showed selectivity toward the atriazine family and a large detection range (0.22 ppb to 3235 ppm).	2009 [167]
Diazinon	AChE	FET	5 ppb	Incubation time 15 min	The extended-gate FET sensor with a ferrocene-modified gold electrode could detect enzyme-catalyzed products without using mediators	2010 [355]
Chloropyrifos	AChE (be)	Voltammetric	51 ppt	Incubation time 10 min	Immobilization of AChE on an oxidized exfoliated graphite nanoplatelet–chitosan cross-linked composite resulted in high affinity, fast response, and high sensitivity	2010 [356]
Monocrotophos	AChE (E. eel)	Fourier transform continuous cyclic voltammetry	2.23 ppb	Response time less than 70 s	The combination of MWCNTs and AuNPs promoted the electron transfer and catalyzed the electrooxidation of thiocholine, resulting in high sensitivity and a fast response time (less than 70 s).	2010 [357]
Dimethoate	AChE (E. eel)	Amperometric	0.46 ppb	Incubation time 8 min	Enzyme was immobilized on a MWCNT–β-CD composite modified glassy carbon electrode	2010 [265]
Carbofuran	AChE	Amperometric	0.88 ppb	Incubation time 6 min	The AChE/dendrimer polyamidoamine–Au/CNT multilayer modified electrode provided a favorable environment for the immobilization of AChE	2010 [358]

Table 2 (continued)

Pesticides	Bioreceptor	Detector	LOD	Detection time	Comments	Year/reference
					and also improved the electrocatalytic characteristics and electron transfer speed	
Methyl parathion	Methyl parathion degrading enzyme	Amperometric	1.0 ppb	10 min	Methyl parathion degrading enzyme was immobilized on an AuNP/MWCNT electrode through a CdTe QD covalent attachment. This approach allowed loading of more enzymes through CdTe QDs and resulted in a remarkably low LOD owing to synergistic effects of MWCNTs and AuNPs. The biosensor showed high selectivity toward pesticides containing a P–S bond and can be potentially reused and is suitable for continuous monitoring	2010 [281]
Chlorpyrifos	PATP/AuNP	Amperometric	115.69 ppb	Incubation time 10 min	The combination of surface molecular self-assembly with electropolymerization of *p*-aminothiophenol on the larger area of an AuNP-modified electrode produced a high ratio of imprinted sites and improved the sensitivity and selectivity	2010 [152]
Paraoxon	BChE	Amperometric	1.38 ppb		The combination of magnetic bead immunocapture-based enzyme activity assay and competitive immunoassay provided a sensitive, rapid, and portable method for biomonitoring of OP compounds	2011 [269]
Carbaryl	AChE (E. eel)	Amperometric	0.7 ppb	Incubation time 2 min	The immobilized AChE on a CdS-decorated graphene nanocomposite possessed higher enzymatic activity and high affinity for the substrate, and the integration of the CdS–graphene nanocomposite made the carbaryl contact with the active site of AChE much more easily, thus reducing the incubation time (2 min)	2011 [268]
Parathion	OPH	Amperometric	145.6 ppb	~10 min	Time-resolved differential pulse stripping voltammetry with a disposable proton-selective sensor allowed the discriminative detection of both parathion and methyl parathion	2011[359]
Monocrotophos	AChE	Amperometric	0.05 ppb	Incubation time 10 min	Mesocellular silica foam functioned as both an enzyme immobilization matrix and a solid phase extraction material for the preconcentration of target molecules; The resulting biosensor showed extremely high sensitivity and LOD	2011 [360]
Trichloropyrindinol	Anti-trichloropyrindinol	Amperometric	0.1 ppb	10 min	The portable immunochromatographic electrochemical biosensors, which combined an electrochemical immunosensor with a lateral-flow test strip, allowed accurate biomonitoring of the metabolite trichloropyrindinol in plasma	2011 [97]

Table 2 (continued)

Pesticides	Bioreceptor	Detector	LOD	Detection time	Comments	Year/reference
Paraoxon	AChE (E. eel)	Amperometric	5.5 ppt	Incubation time 15 min	The first enzymatic assay for detection of AChE activity and pesticides based on the assembly of thiocholine onto Fe_3O_4/AuNP nanocomposites and its oxidation at the electrode surface	2011 [361]

AChE acetylcholinesterase, *AuNP* gold nanoparticle, *BChE* butyrylcholinesterase, *be* bovine erythrocytes, *β-CD* β-cyclodextrin, *ChOx* choline oxidase, *CNT* carbon nanotube, *D. m Drosophila melanogaster*, *DNOC* 4,6-dinitro-*o*-cresil, *EDOT* 3,4-ethylenedioxythiophene, *E. eel Electrophorus electricus*, *EGDMA* ethylene glycol dimethacrylate, *he* horse erythrocytes, *ISE* ion-selective electrode, *LBL* layer by layer, *MAA* methacrylic acid, *MWCNT* multiwalled carbon nanotube, *OP* organophosphorus, *PAAT* poly (3-acetic acid thiophene), *PATP* polyaminothiophenol, *PVA* poly (vinyl alcohol), *QD* quantum dot, *SbQ* styryl-pyridinium groups, *SPE* screen-printed electrode, *SWCNT* single-walled carbon nanotube

complex and depositing a new sensing layer. Development of piezoelectric immunosensors for competitive and direct determination of atrazine [196, 299] was also reported.

The QCM technique takes advantage of a mass-sensitive detector based on an oscillating piezoelectric quartz crystal that resonates at a specific frequency. To obtain high selectivity for analytes of interest, the QCM surface needs to be coated with a sensitive layer that is able to recognize target molecules. The sensitive layer used for detection of pesticides has included AChE [300–302], molecularly imprinted monolayers [148], and specific antibodies [303–306]. It was reported that detection of OP and carbamate pesticides was achieved by measuring the precipitation of an enzymatic reaction product over a QCM electrode [302]. The QCM-based enzyme sensor was based on the fact that oxidation of the 3,3′-diaminobenzidine substrate by a serial reaction of AChE, ChOx, and horseradish peroxidase produced 4,4′-diimino-3,3′-diaminobiphenyl, which was a signal-amplifying precipitate over the QCM electrode. Kim et al. [300] immobilized AChE over the surface of a QCM electrode, resulting in an improved QCM–precipitation sensor. The sensor developed was capable of sensitive detection of EPN and carbofuran, with an LOD of 5 and 0.29 ppb, respectively. QCM-based MIP sensors were also developed for measuring parathion [133], pirimicarb in vegetables [299], imidacloprid and thiacloprid in aqueous solution and in celery juice [154], and atrazine [156, 307]. Bi and Yang [154] proposed a two-template-imprinted method for detecting and differentiating two similar pesticides, imidacloprid and thiacloprid. In this method, templates (thiacloprid and imidacloprid) were preadsorbed on QCM sensor chips, and QCM sensor chips with preadsorbed templates were then exposed to alkanethiols, which self-assembled around the preadsorbed templates. The surface was then rinsed thoroughly with pure ethanol and deionized water sequentially to remove thiacloprid or imidacloprid and two different molecularly imprinted binding sites were created. A single QCM sensor decorated with two-dimensional molecularly imprinted monolayers could respond to 2.56 ppm imidacloprid and 2.53 ppm thiacloprid in celery juice in a real-time manner.

Rapid screening assay

There has been increased interest in development and implementation of rapid screening methods for detection of pesticide residues. The lateral-flow immunochromatographic assay has become an increasingly practical tool in pesticide analysis because it combines several benefits, including a user-friendly format, short assay time, and cost-effectiveness. Such strip-based immunoassays do not need fussy operations such as incubation, washing, and enzymatic reactions during signal generation, which distinctly

shorten the detection time. Especially, the results can be read directly by the naked eye, which makes the assay convenient. These characteristics make it well suited for on-site screening of pesticides in food and the environment [101, 308–319]. For example, Kaur al. [320] reported a lateral-flow-based dipstick immunoassay using a novel hapten–protein–gold conjugate for determination of atrazine in water samples (Fig. 8). The dipstick could visually screen atrazine-contaminated water samples down to the 1-ppb level with an analysis time of 5 min. An immunostrip test for thiabendazole was developed based on an indirect competitive principle using carbon particles as a label [308]. The optimized test was accomplished within 10 min and the semiquantitative visual evaluation of the immunostrip provided an LOD of 0.25 ppb. The linear working range of the standard curve for quantitative evaluation was 0.11–4.13 ppb. When applied to analysis of spiked fruit juice by the direct dilution method, without sample extraction or cleanup, the strip assay was able to determine a thiabendazole concentration of 50 ppb with acceptable accuracy and reproducibility. Morais et al. [104] developed multiplexed microimmunoassays for five pesticides by using a digital versatile disc (DVD) as an analytical support. In this system, the detection principle was based on capture of attenuated analog signals with the disk drive that were proportional to the optical density of the immunoreaction product. For that, a set of protein–hapten conjugates with pesticides and antibiotics were microarrayed on the polycarbonate face of DVDs, and a pool of specific antibodies, gold-labeled secondary antibodies, and silver amplification were used for developing the assays. The multiplexed assay achieved LODs of 0.06, 0.25, 0.37, 0.16, and 0.10 ppb, and dynamic ranges of two orders of magnitude for atrazine, chlorpyrifos, metolachlor, sulfathiazole, and tetracycline, respectively.

Zou et al. [96] labeled 3,5,6-trichloropyridinol (TCP) with semiconductor nanoparticles and developed an immunochromatographic fluorescent biosensor for detection of TCP. The principle of the sensor was based on competitive binding between the various amounts of analytes and nanoparticle–TCP conjugates onto a fixed amount of antibody (TCP antibody) on the test zone (Fig. 9). It was demonstrated that this immunosensor could detect as little as 1.0 ppb TCP in 15 min, with a linear range from 1 to 50 ppb.

ChE-based colorimetric sticks or strips for class-specific detection of OP and carbamate pesticides have also been developed [321–326]. Such assays using colorimetric strips or papers to detect inhibition by color development with the naked eye allow qualitative and semiquantitative detection of pesticides in the field. For example, No et al. [322] developed a dipstick-type assay for detection of paraoxon. AChE was coated on a Hybond N^+ membrane. The proposed assay involved incubation of an AChE-coated strip in the pesticide solution followed by incubation of the sample-treated strip in a chromogenic substrate solution. The color intensity was monitored by the naked eye or a reflectometer. This dipstick assay could detect paraoxon at parts per billion levels. Hossain et al. [326] reported a paper-based assay in which AChE was printed within biocompatible sol-gel-

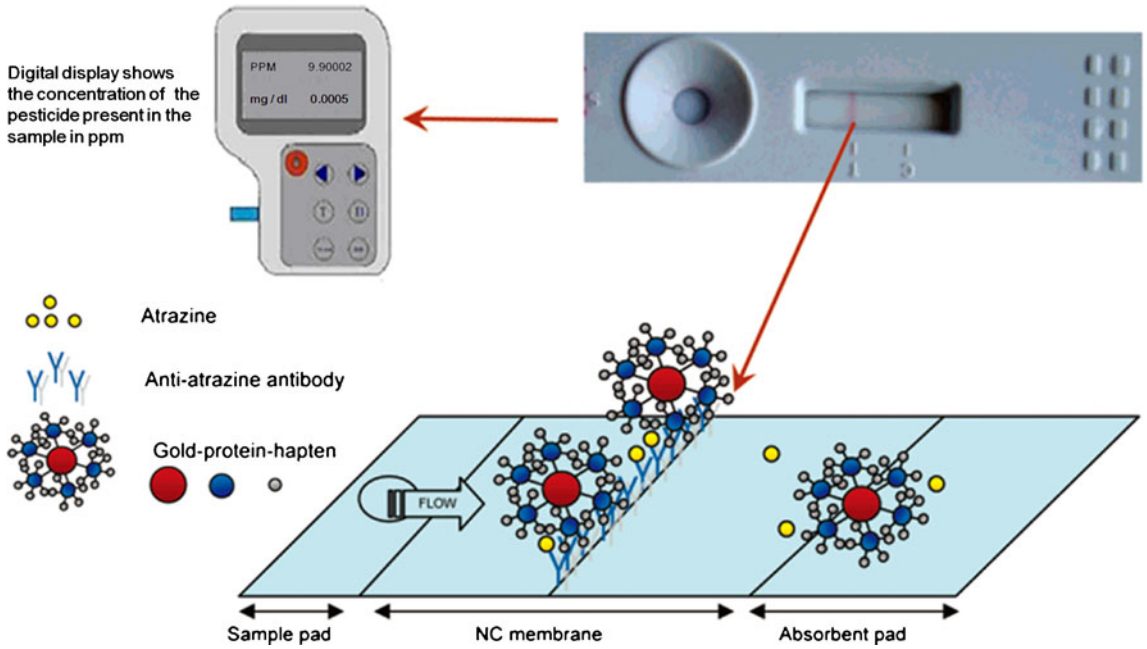

Fig. 8 Immunochromatographic dipstick employed in the assay described, showing cross-reaction and the assay format. *NC* nitrocellulose. (Reproduced with permission from [320]. Copyright 2007 American Chemical Society)

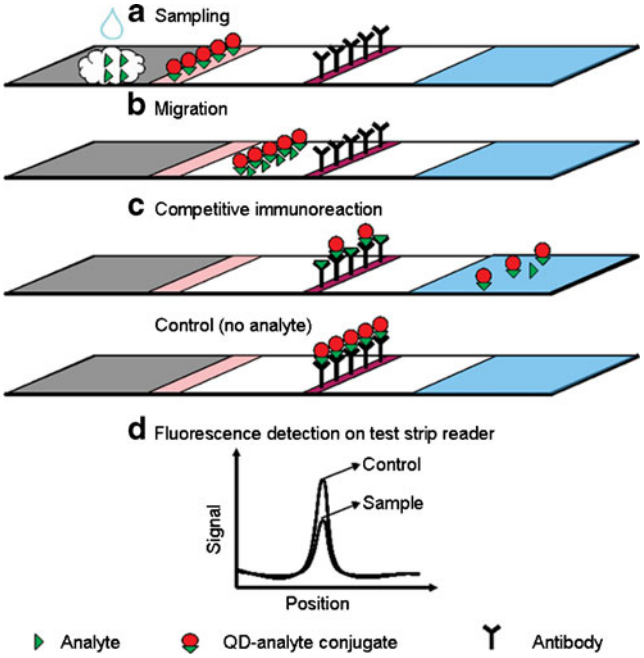

Fig. 9 The principle of the fluorescent immunochromatographic test strip assay. **a** Aqueous sample containing analytes is applied to the sample zone. **b** Analytes migrate together with nanoparticle-conjugated competitors toward the other end of the test strip by capillary force. **c** Competitive immunoreactions among analytes, conjugated competitors, and antibodies. Excess conjugated competitors and analytes continue to migrate toward the absorption pad. In a control assay (no analyte), the nanoparticle-conjugated competitors bind fully to the antibodies in the test zone. **d** Fluorescence detection on the test strip reader. *QD* quantum dot. (Reproduced with permission from [96]. Copyright 2010 American Chemical Society)

derived silica layers onto paper supports. The assay provided good LODs of 27.5 ppb for paraoxon and 0.31 ppb for aflatoxin B_1 and rapid response times (less than 5 min). One of the disadvantages of the above-mentioned assay systems is the requirement of the addition of an external reagent. Hossain et al. [321] developed a fully integrated reagentless paper-based lateral-flow device in which biocompatible sol-gel-derived silica was employed as an entrapment agent to deposit not only the enzyme but also all other reagents required onto a paper support via piezoelectric inkjet printing. The assay strip was composed of sensing and substrate zones on a paper support, in which AChE and a chromogenic substrate, indophenyl acetate, were entrapped using biocompatible sol-gel-derived silica inks (Fig. 10). The proposed assay involved lateral flow of a pesticide-containing solution to the sensing zone followed by inverted lateral flow of the indophenyl acetate substrate to the sensing zone to initiate enzyme-catalyzed hydrolysis, causing a yellow-to-blue color change. The strip was able to detect pesticides at the nanomolar level. The assay system showed a negligible matrix effect with pesticide-spiked milk and apple juice samples.

Food and environmental applications

Despite the elevated number of publications on biosensors for pesticides, most of these systems reported in the literature have been tested on standard solutions rather than real samples. The absence of practical examination makes it difficult to fully evaluate the usefulness of these devices for real sample monitoring. Fortunately, some articles have focused on demonstrating the potential applications of the sensors to real samples, mainly in water samples [179], but also in biological fluids [132], in fruit samples [205, 214], or OP pesticide contamination in air [327]. Table 3 summarizes the results published during the last 10 years showing applications to food and environmental samples. As can be seen, the practical application of various biosensors is still in the early stage, and many obstacles remain to be overcome. A main challenge seems to be that the most used biorecognition elements are not sufficiently selective or sensitive in the real environment.

Correspondingly, commercialization of these devices for real practical applications is in its infancy. Encouragingly, several types of assays for pesticides are now commercially available. Among the currently available commercial enzymatic testing kits for pesticides are the OP-Stick Sensor (Protein Biosensor, Toulouse, France), the Neuro-IQ Tox Test Kit (Aqua Survey, Flemington, PA, USA), an organophosphate/carbamate screen kit (Abraxis, Warminster, PA, USA), Eclox pesticide strips (Severn Trent Services, Fort Washington, PA, USA), and a pesticides in water test kit (PRO-LAB,Weston, FL, USA). We also notice that the time required for a test and the sensitivity of these products still have room for improvement in order to meet the requirements for the fast and sensitive trace analysis of pesticides in real samples. For example, Protein Biosensor's OP-Stick Sensor is an AChE-based colorimetric assay designed for detecting pesticide residues in water, soil, and food. The assay provided an LOD of 100 ppm for aldicarb. Including setup and operation, the time required for a test was approximately 1.5–2 h.

Challenges, strategies, and future perspectives

In the past two decades, considerable advances have been made in the detection of pesticides using sensors based on biological macromolecules (enzymes and antibodies), artificial macromolecules (MIPs), and whole cells. The main analytical parameters, e.g., sensitivity, selectivity, and stability, have been significantly improved by using different transducers, immobilization approaches, and new materials. Despite the promise of pesticide biosensors, they do have certain limitations, and improvements are still required in the design of biosensors before they become widely accepted in environmental control.

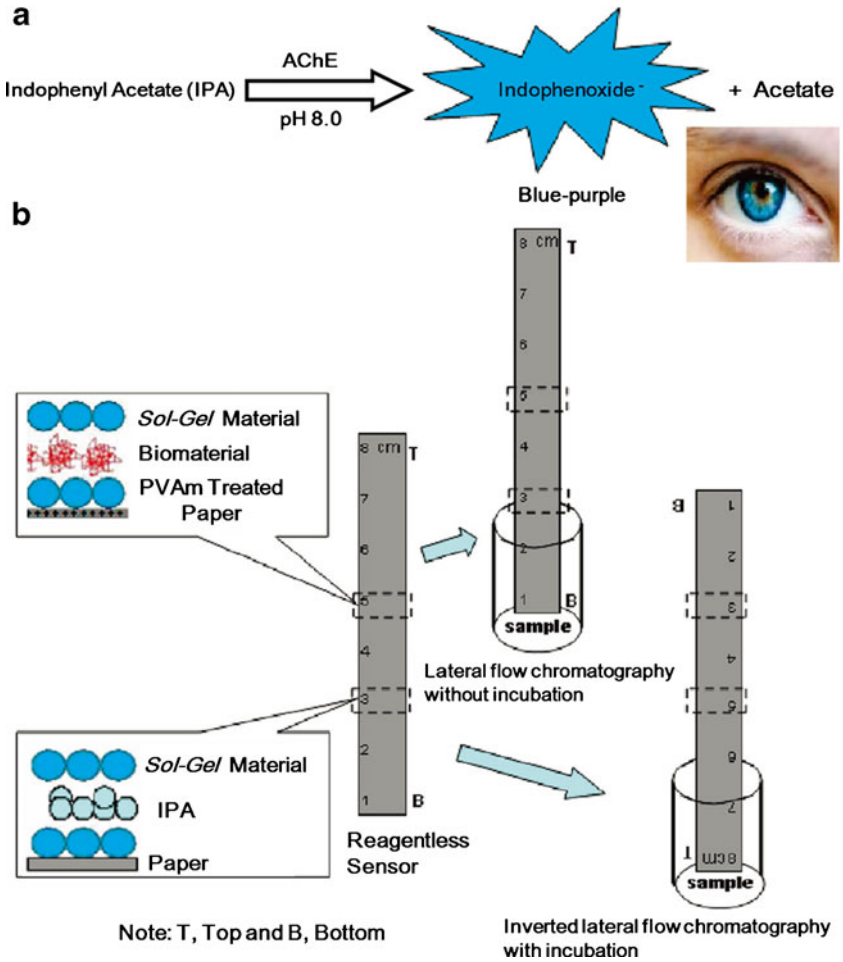

Fig. 10 **a** The detection principle of the indophenyl acetate (*IPA*)-based colorimetric assay. AChE hydrolyzes the red-yellow substrate IPA under slightly basic conditions (pH 8.0) to form indophenoxide anions, which are blue-purple. **b** Development of the reagentless bioactive paper-based lateral-flow sensor in which AChE and IPA are entrapped in the regions marked by the *dashed boxes* on a Whatman no. 1 paper strip (1 cm×10 cm) following the sequent deposition of polyvinylamime (*PVAm*)/silica/AChE/silica and silica/IPA/silica, respectively, by the use of either inkjet printing or overspotting. The sensor can be used in two different ways: (1) directly (normal lateral-flow-based assay) without incubating the contaminated sample and (2) inverted lateral-flow-based assay with incubation of the sample. (Reproduced with permission from [321]. Copyright 2009 American Chemical Society)

Enzyme-based sensors (whether OPH-based or AChE-based biosensors) allow detection of broad families of pesticides and offer a general toxicity index, without providing specific information about a particular pesticide. They are both very sensitive and selective in their approach to detecting pesticides. OPH-based sensors offer additional advantages over AChE-based systems owing to possible reuse. However, these biosensors have lower sensitivity and higher LODs than ChE-based biosensors. In many cases, enzyme-inhibition-based biosensors have high sensitivity and could be the basis for relatively simple and cost-effective procedures when the analyte is present alone. However, enzyme-inhibition-based biosensors have three major drawbacks:

1. One of the unique features of enzyme-inhibition-based biosensors is that the inhibition is irreversible. Various oximes, such as 1,10-trimethylene bis(4-formylpyridinium bromide) dioxime [328] and pralidoxime [98, 244, 256, 258, 266, 268, 329–334], have been tried as reactivators for AChE- or BChE-based sensors. However, regeneration is always partial and takes time, ranging from 15 min to overnight, which obviously depends on the degree of inhibition and the type of pesticides. Therefore, most studies are focused on the development of disposable biosensors.
2. The principle of AChE- or BChE-based sensors is an enzyme inhibition mechanism, and therefore such biosensing systems require lengthy incubation times to allow interaction of the enzyme and analyte. Furthermore, the enzyme activity is key for these biosensors. How to keep the enzyme activity during storage and detection processes is a major challenge.
3. Since a number of pesticides have a similar mode of action affecting the activity of the same enzyme, most enzyme-inhibition-based biosensors can give a sum parameter of the inhibition efficiency without any qualitative or quantitative information about the individual analytes. The lack of selectivity means enzyme-inhibition-based biosensors can only be applied for general screening of natural samples.

The approaches for detection and discrimination of pesticides were investigated by several groups. Simonian et al. [52] used a combined recognition/discrimination strategy based on the joint action of AChE and OPH, and demonstrated the feasibility of distinguishing OP pesticides from

Table 3 Some examples of biosensors for determination of pesticides in real samples

Transducer	Bioreceptor	Pesticide analyzed /LOD	Applications in real samples	Comments	Year/reference
Amperometric	Mutant AChE	Parathion/20 ppb	Infant food	Spiked sample mixed with phosphate buffer and preincubated with P450 BM-3 before detection. Overall assay time was 95 min	2004 [362]
Amperometric	AChE (E. eel)	Paraoxon/0.145 ppb	Lake water	Incubation time 30 min.	2005 [347]
Piezoelectric	AChE (E. eel)	Diisopropyl fluorophosphate/ 0.02 ppb	River water	The sensor could be regenerated and used for 40 measurements. The sample needs only minimal preparation (ultrafiltration). Total measurement time was 60 min	2005 [363]
Photonic crystal sensing	AChE	Parathion/0.001 ppt	Water	The sensor had the lowest LOD and can overcome the high ionic strength interference from field samples. Overall assay time was 15 min	2005 [4]
Amperometric	BChE (he)	Chlorpyrifos-methyl oxon/0.5 ppb	Tiber water	Heavy metals had no interference effect, but detergents resulted in enzyme decay; the medium exchange method and a washing step were required to circumvent the interference of detergents	2006 [364]
ELISA	Anti-acetamiprid	Acetamiprid/53 ppt	Peach, apple, strawberry, and eggplant	The sample was extracted with methanol, and simple dilution of sample extracts with water was used to eliminate matrix interferences. The assay time was more than 70 min	2006 [120]
Visual	AChE (bs)	Diazinon oxon/0.1 ppm	Apple and orange juice	The apple sample needed to be extracted with hexane to avoid interference and the total time was about 1 h. Since the chip used free enzyme, it had good stability (6 months at 4 °C)	2007 [365]
Amperometric	AChE (D. m)	Dichlorvos/0.04 ppb	Carbonated beverage, beer, orange juice, and milk	No sophisticated sample pretreatment was required and the beverages showed no interference with the sensor	2008 [366]
ELSA	Anti-dinitroaniline	2,6-dinitroaniline/0.61±0.08 ppb	Tap water and soil sample	The total assay required 1 h 50 min and dilutions of the soil extracts were required. Blanks of the soil samples (without spikes) showed some background	2008 [119]
Visual	AChE (E. eel)	Paraoxon/0.28 ppb	Beverages and food samples	The paper-based solid-phase biosensor is able to detect pesticides without the use of any external reagents in short time (~5 min) and showed negligible matrix effects in detection of pesticides in spiked milk and apple juice samples	2009 [321]
Amperometric	AChE (E.eel)	Methyl parathion and chlorpyrifos/0.26 ppt	Spiked river water	The sample was filtered and centrifuged. The biosensor inhibited by pesticides could reacquire 90 % of the original activity after incubation with pralidoxime for 15 min	2009 [266]
QCM	Antibody	Trichloropyrindinol/7 ppb	Orange and apple juice samples	Samples were analyzed without any pretreatment other than dilution. Each complete assay cycle took 20 min. Good sensitivity, specificity, and reusability were achieved	2009 [102]
Potentiometric	Bacteria and AChE (be)	Chlorophos/0.26 ppb	Contaminated milk	Sample preparation followed the Boehringer-Mannheim protocol, and then the filtrate was treated with glucose	2009 [95]

Table 3 (continued)

Transducer	Bioreceptor	Pesticide analyzed /LOD	Applications in real samples	Comments	Year/reference
				oxidase to prevent glucose interference. The biosensor was reusable and the response time was 200 s	
ELISA	Anti-metolcarb	Metolcarb/1.2 ppb	Apple juice, orange juice, cabbage, and cucumber	Dilution of extracts with buffers was necessary to reduce the matrix interferences. The assay needed 1.5 h.	2009 [108]
Amperometric	AChE (he)	Paraoxon/27.5 ppt	Saliva samples	The portable, rapid, and sensitive device based on a CNT sensor could be regenerated. The inhibition time was 30 min; no complicated sample pretreatment was necessary	2009 [367]
Visual	Antibody	Paraoxon/0.16 ppb	Water	The analytical procedure only needed sample dilution to adjust the pH and ionic strength. The time taken for a competitive multiplexed microimmunoassay was almost 30 min	2009 [104]
Amperometric	Polyethyleneimine/SiO_2	Parathion/3 ppb	Cucumber and cabbage samples	Simple sample treatment without extraction or preconcentration	2009 [63]
Visual	Antibody	Thiabendazole/0.25 ppb	Fruit juices	Matrix interferences were avoided by simple dilution of samples. An optimized test was accomplished within 10 min	2010 [308]
Amperometric	AChE	Carbofuran/0.88 ppb	Onion, lettuce, and cabbage	Sample extracts were directly detected without extraction or preconcentration. The incubation time was short, i.e, 6 min	2010 [358]
Fluorescence	Anti-bromopropylate	Bromopropylate/1.5 ppb	Orange juice	The assay did not require the separation steps for a conventional immunoassay and was accomplished within 5 min. It could be suitable for multiple analytes	2010 [131]
Fluorescence	AChE(E. eel) and ChOx	Paraoxon/0.76 ppt	Apple	Dilution of the sample extract was required and the total time was about 20 min	2011 [205]
Immunoamperometric	BChE	Paraoxon/1.38 ppb	Human plasma samples	There was less interference because magnetic beads were used for separation of analytes from human plasma samples. The total time was 1 h 5 min (1 h incubation+5 min detection)	2011 [269]
Fluorescence	AChE(E. eel)	Paraoxon/2.9 ppt	Apple and vegetables	Dilution of the sample extract was required and the total time was about 20 min. The approach could be used for multianalyte analysis	2011 [214]
Amperometric	AChE	Monocrotophos/0.05 ppb	Garlic samples	With the reactivation procedure, this biosensor could be repeatedly used for 6 cycles. The incubation time was 10 min	2011 [360]
Visual	Esterase 2	Paraoxon/2.75 ppb	Fruit juices	The sensor showed high specificity toward paraoxon and high stability toward temperature, organic solvents, and pH, which allowed its use in extreme conditions	2011 [368]
Fluorescence	AChE (E. eel)	Paraoxon/1.0 ppt	Tap water and lake water	Simple sample pretreatment, but the assay took more than 75 min	2011 [369]
SPR	MAA/TRIM	Acephate/7.86×10^{-3} ppt	Apple and cole samples	The MIP film exhibited high selectivity for acephate. Extraction of the real samples was required	2011 [165]

bs bovine serum, *TRIM* Trimethylolpropane trimethacrylate

non-OP pesticides. In another study, since the structure of ChE itself can define its affinity for different OP compounds, the use of genetically modified mutant enzymes to construct biosensors is expected to increase both the selectivity and the sensitivity of the sensors. In addition to mutant enzymes, the use of multielectrode biosensors modified with native and recombinant variants of AChE coupled with chemometric data processing was developed for discrimination of binary mixtures of organophosphates and carbamates [335]. The assay duration was 40 min and the LOD for paraoxon and carbofuran in drinking water in separate analyses was 0.5 ppm. For inhibition analysis of binary mixtures, the sensor had a resolution error of 0.4 ppm for paraoxon and 0.5 ppm for carbofuran, respectively. In the same way, Crew et al. [295] used genetically modified *Drosophila melanogaster* AChE for determination of OP pesticides. In this work, screen-printed carbon electrodes modified with cobalt phthalocyanine were used as base transducers. However, the practical application is hampered by the fact that AChEs from various sources are not readily available because of the difficulties in isolating purifying them. Moreover, interestingly, numerous investigations demonstrated that subtle changes in the surface characteristics (e.g., surface wettability) during the immobilization process affected the orientation of the immobilized enzyme, its stability, and its affinity and specificity for some OP compounds [214, 336]. Thus, improvement of sensor performance may be expected by tuning the structures of the biosensors and therefore manipulating their selectivity for different types of pesticides. Our group recently described development of an optical biosensor for reliable detection of the total content of OP mixtures in foodstuffs [205]. The basic element of the biosensor was an ordered nanostructured film composed of AChE, ChOx, and CdTe QDs, which was fabricated by using a layer-by-layer assembly technique. The introduction of bienzymes not only lowered the LOD for OP compounds, but also improved the capability for multianalyte detection. This proposed biosensor was proved to be suitable for accurate detection of the total concentration of binary mixtures of paraoxon with dicholovos or parathion.

Furthermore, immobilization of enzymes is a key step in optimizing the analytical performance of enzyme-based biosensors. An effective immobilization method or matrices might improve not only the stability and sensitivity of the sensor but also the response time. Various methods and new materials such as nanomaterials have been employed to immobilize enzymes and considerably improve the stock stability of the enzyme. In addition, pesticides are generally hydrophobic compounds and their extraction and concentration from water or agricultural products are based on organic solvents. Therefore, developing an enzyme sensor that can work in organic media seems promising.

From an application standpoint, immunosensors can offer real-time, continuous monitoring of pesticides in a matrix. They are particularly useful for polar or water-soluble pesticides. They also can be significantly faster than some conventional methods, because the cleanup step can be avoided or shortened for aqueous samples (such as juice and milk) and for many fruits and vegetables. Despite these advantages, the use of immunosensors for monitoring pesticide residues in food has been limited by a number of factors:

1. It is necessary to know what compound is to be measured and to select appropriate antibodies, because immunosensors are compound-specific. Therefore, they are not suitable for multiple-residue analysis.
2. The crucial step for immunosensors is to generate appropriate antibodies with the desired specificity. However, the characteristics of pesticides, in some cases, may also preclude the use of immunosensors. For example, for those pesticides that are very small molecules or have nonrigid structures, it may not be possible to develop antibodies. In addition, immunosensors may not work well in certain foods.
3. Immunoassays may not be as sensitive as conventional methods for some compounds, and they can have lower levels of reproducibility. The recent development of antibody technologies and protein engineering provides further impetus in this area, because more specific antibodies could be generated in a more cost-effective manner.

The introduction of MIPs and nucleic acid aptamers into pesticide detection increases stability and offers new possibilities. One of the important advantages of these artificial molecules is that they are cheap, fast-acting, and easily produced compared with antibodies [337, 338]. By rational design of the structure and optimization of the selection conditions, it is possible to obtain very sensitive and specific recognition elements [339]. Another important advantage of these artificial molecules is their improved stability. They allow the use of a wider variety of buffers or solvents, simplifying sample preparation or making it suitable for determination or extraction of pesticides that are poorly soluble in water. The analytical potential endowed by MIPs and nucleic acid aptamers may provide solutions to some of the problems encountered in the measurement of pesticides [340]. Meanwhile, we notice that some novel nanomaterials have recently been coupled with versatile aptamers and MIPs for the design of a sensing platform with high selectivity and sensitivity [338, 339]. However, the unique properties of aptamers have not yet been fully exploited for development of pesticide biosensors. The main limitation imposed by the use of aptamers lies in the fact that screening of appropriate aptamers is difficult and tedious [173, 340].

Although only a fraction of MIPs and nucleic acid aptamers have been reported until now, we expect acceleration in this field as the aptamer field progresses.

Whole-cell biosensors offer the advantages of being cheap, having improved stability, and ease of immobilization. Despite the promise of whole-cell biosensors, they do have certain limitations. For example, the absence of specificity is their main disadvantage, which could be overcome by using genetically engineered bacteria with lux fusions to inducible genes and operons.

Furthermore, most studies are focused on detection of OP pesticides, carbamate insecticides, and triazine herbicides, which are used in very large quantities. Meanwhile, there is increasing effort to use other families of pesticides with lower mammalian toxicity, such as neonicotinoids, insect growth regulators, and various types of biopesticides, including biochemical pesticides, microbial pesticides, and plant-incorporated protectants. For example, the neonicotinoids are beginning to dominate the market because they have reduced toxicity compared with previously used OP and carbamate insecticides. Unfortunately, recent studies demonstrate that they are also persistent in the environment and can accumulate in pollen and nectar of the treated plants. Moreover, they may adversely affect human health, especially the developing brain. Therefore, developing biosensors for these pesticides is necessary. As an example, neonicotinoids have strong affinity for insect nicotinic acetylcholine receptor, and thus nicotinic acetylcholine receptor could be used as the biological receptors for their detection.

Conclusion

Owing to scientific and public concern about environment pollution, significant progress has been achieved toward the development of relatively simple, quick, and cost-effective detection methods for pesticide residues in the environment and foods. Biosensors offer valuable opportunities to resolve the problems encountered in the measurement of pesticides by traditional chromatographic methods. However, the most important challenges for transfer of pesticide biosensors from the pristine research laboratory to real-life and commercial applications are related to the stability and sensitivity of the biological recognition elements, to the reproducibility of the biosensors, and to the variability of real samples (especially considering that more than 800 active ingredients are present in a wide range of commercial pesticides). The use of genetically modified enzymes, highly specific antibodies, microbial cells, aptamers, and MIPs for the design of biosensors can be an effective way to improve sensor selectivity. The use of nanostructured materials and microfabrication/nanofabrication technologies might give these biosensors some advantages, for instance, higher sensitivity, faster responses, and smaller instrument and sample sizes [338, 339]. In the future, more emphasis is required on the development of a simple, portable, and reliable biosensing system for fast and automatic analysis of pesticides in real samples.

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