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

The contamination of soil and food due to pesticides has caused a serious concern; therefore, to watch over the safety of marketed food supplies. International organizations (e.g., Food and Agriculture Organization) regulate their maximum residue levels on foods and agricultural commodities. Contamination of ground and surface water by pesticide residues had been regarded as transitory because the focus was on OP pesticides, which were of very low water solubility and had a strong tendency to attach to particulate matter. However, the information accumulated during recent years about generally more soluble organochlorine pesticides and other herbicide compounds has shown the presence of pesticide residues in both surface and ground water. Consequently, policies have been made to reduce contamination of ground and surface water.
Regulatory limits and guideline levels have also been introduced for permissible residues in drinking water [8].

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

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

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

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

Biosensors

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

Basic principle of AChE biosensors

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

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

AChE immobilization

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

Physical adsorption

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

Merits: No damage to enzyme, simple and cheap way of immobilization, no chemical change of the support, and reversible to allow regeneration of free enzyme.

Demerits: Leakage of enzyme, nonspecific binding, overloading of the enzyme on support, short response time, poor operational and storage stability, and sensitive to changes in pH, temperature, and ionic strength [36].

Physical entrapment

Physical immobilization methods such as entrapment in sol–gel matrices and lattice of a polymer matrix or membrane have also
been used for AChE electrodes. It has been done in such a way as to retain protein while allowing penetration of substrate.

**Merits:** One-step procedure at ambient or low temperature, no damage to enzyme, simple and cheap way of immobilization, no chemical change of the support, and suitable for a large variety of bioreceptors.

**Demerits:** Leakage of enzyme, nonspecific unstable immobilization, many biocompatible polymers available, and problems of reproducibility and control of pore size and diffusion barriers [25,37–40].

---

**Covalent coupling**

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

**Merits:** Absence of diffusion barriers, short response time, no enzyme leakage, and wide range of choices for selecting carrier material (making the method flexible with specific chemical and physical properties).
Demerits: High amount of enzyme, possible denaturation, and expensive and complicated procedures [41–45].

Self-assembled monolayer

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

Merits: High degree of structural order, nanometer size, molecular recognition properties, ease of preparation, and diversity of terminal functionalities.

Demerits: Possible electrode fouling, complex, and difficult to reproduce [47,48].

Oriented immobilization

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

Merits: Reusable surface, low amount of enzyme, and controlled and orientated immobilization [49].

Demerits: Requires the presence of specific groups in the bioreceptor molecule (e.g., histidine, biotin, concanavalin A).

Electropolymerization

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

Merits: Ability of coating electrodes, which have a small or non-uniform surface, to control the polymer thickness, prevention of interferences or electrode fouling. In addition, many polymers behave as mediators or are used for the immobilization of a mediator [50,51].

Demerits: Occurs only on conducting substrates (limiting the types of surfaces that can be electropolymerized).

Classification of AChE biosensors

Membrane-based AChE biosensors

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

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA–SbQ membrane/Pt electrode</td>
<td>Amperometric</td>
<td>Entrapment</td>
<td>$7.2 \times 10^{-5}$, 1.88, and 0.049</td>
<td>NR</td>
<td>Paraoxon, maneb, and thifensulfuron methyl</td>
<td>30</td>
<td>30</td>
<td>[52]</td>
</tr>
<tr>
<td>Nylon and cellulose nitrate membrane/pH electrode</td>
<td>Potentiometric</td>
<td>Crosslinking with glutaraldehyde</td>
<td>0.018 and 0.077</td>
<td>$5.0 \times 10^{-7}$–$2.5 \times 10^{-5}$</td>
<td>TrichlorfonCo-Ral</td>
<td>15</td>
<td>30</td>
<td>[54]</td>
</tr>
<tr>
<td>Glass/sol–gel indicator/polyvinylidene fluoride membrane</td>
<td>Fiber-optic</td>
<td>Crosslinking with glutaraldehyde</td>
<td>0.53 and 0.023</td>
<td>0.54–39.8 and 0.022–0.13</td>
<td>CarbarylDichlorvos</td>
<td>10</td>
<td>21</td>
<td>[55]</td>
</tr>
<tr>
<td>Poly(2-hydroxyethyl methacrylate) membrane/oxygen electrode</td>
<td>DO metric</td>
<td>Crosslinking with glutaraldehyde</td>
<td>1.19</td>
<td>0.05–2.62</td>
<td>Aldicarb</td>
<td>5</td>
<td>2</td>
<td>[56]</td>
</tr>
<tr>
<td>Cellophanemembrane/AuE Hybrid mesoporous silica membrane/Pt electrode</td>
<td>Amperometric</td>
<td>Entrapment</td>
<td>1.45 and 1.2 $\times 10^{-3}$</td>
<td>1.45–7.26 and 1.0 $\times 10^{-3}$–0.3</td>
<td>Paraoxon</td>
<td>15</td>
<td>NR</td>
<td>[57]</td>
</tr>
</tbody>
</table>

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

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

**Polymeric matrix-based AChE biosensors**

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

**AChE biosensors based on nonconducting polymer matrices**

Supports used for immobilization of AChE: Polyvinyl alcohol-bearing styrylpyridinium groups (PVA–SbQ) membrane [52], polycrylamide membrane [53], nylon and cellulose nitrate membrane [54], glass/sol–gel indicator/polyvinylidene fluoride membrane [55], poly(2-hydroxyethyl methacrylate) membrane [56], cellophane membrane [57], hybrid mesoporous silica membrane [58], and poly-(acrylonitrile–methylmethacrylate–sodium vinylsulfonate) (PAN) membrane [59,60].

**Merits:** Artificial membranes have high selectivity for certain bioelements and amplify responses because of their higher degree of flexibility, mechanical durability, wider pH range for use, and higher specific activity.

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

**AChE biosensors based on conducting polymer matrices**

Supports used for immobilization of enzyme: Polyacrylamide membrane/pH electrode [53], polylethyleneimine (PEI)-coated GCE [65], PEI/SPE [41], mercaptobenzothiazole/polyaniline (PANI)/Au electrode [66], PANI/CNTs wrapped with single-stranded DNA (ssDNA)/Au electrode [67], AuNP–polypyrrole (PPy) nanowire composite film modified GCE [68], PPy and PANI copolymer doped with MWCNTs/GCE [69], ZnS, and polyn(in-dole-5-carboxylic acid)/Au electrode [70].

**Merits:** Polymer matrices as supports have enhanced speed, sensitivity, and versatility in diagnostics of desired analytes. Conducting polymers are the conjugated polymers that can be synthesized by chemical methods as well as electrochemical methods, provide easy moduliation of various properties (e.g., film thickness, conductivity, functionalization, use of various supporting electrolytes, ability to serve as an electrochemical transducer itself). Additional merits include entrapping of enzyme molecules during electropolymerization in one step and also uniform covering of the surface of substrate electrodes of any shape or size by polymer film [71,72].

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

**Sol–gel-based AChE biosensors**

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

Sol–gel supports used for immobilization of enzyme: Sol–gel crystals derived from tetramethyl orthosilicate (TMOS) [73], sol–gel film on a glass cap [74], silica sol–gel [37], TMOS sol–gel film [40,75], chroinoonophore (ETH5294) doped sol–gel film [76],
Table 3

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNTs/PAN membrane/Pt electrode</td>
<td>Amperometric</td>
<td>Affinity bonds using concanavalin A</td>
<td>5.0 x 10^-6</td>
<td>3.6 x 10^-5 3.6 x 10^-5</td>
<td>Paraoxon</td>
<td>20</td>
<td>120</td>
<td>[59]</td>
</tr>
<tr>
<td>PAN/AuNPs/Pt electrode</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>0.026 x 10^-5</td>
<td>3.6 x 10^-7 3.6 x 10^-4</td>
<td>Paraoxon</td>
<td>20</td>
<td>30</td>
<td>[60]</td>
</tr>
<tr>
<td>MSF-PVA/GCE</td>
<td>Amperometric</td>
<td>Entrapment</td>
<td>0.2 x 10^-3</td>
<td>0.2 x 10^-3 44.8 x 10^-3</td>
<td>Monocrotophos</td>
<td>10</td>
<td>NR</td>
<td>[63]</td>
</tr>
<tr>
<td>PVA-SbQ polymer/SPE</td>
<td>Amperometric</td>
<td>Entrapment</td>
<td>1.91 x 10^-2</td>
<td>NR</td>
<td>Paraoxon and chlorpyrifos-ethyl oxon</td>
<td>10</td>
<td>NR</td>
<td>[38]</td>
</tr>
<tr>
<td>PVA-SbQ membrane/Pt electrode</td>
<td>Amperometric</td>
<td>Enzyme</td>
<td>7.2 x 10^-3 0.18, and 0.049</td>
<td>NR</td>
<td>Paraoxon, maneb, and thifensulfuron methyl</td>
<td>30</td>
<td>30</td>
<td>[52]</td>
</tr>
<tr>
<td>PAMAM-gold/CNTs/GCE</td>
<td>Amperometric</td>
<td>Electrostatic interaction</td>
<td>4.0 x 10^-3 4.8 x 10^-2 9.0 x 10^-2</td>
<td>NR</td>
<td>Carbofuran</td>
<td>9</td>
<td>21</td>
<td>[64]</td>
</tr>
</tbody>
</table>

Note: NR, not reported.

Table 4

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyanacrylamide membrane/pH electrode</td>
<td>Amperometric</td>
<td>Crosslinking</td>
<td>3.62 x 10^3</td>
<td>NR</td>
<td>Dichlorvos</td>
<td>30</td>
<td>50</td>
<td>[53]</td>
</tr>
<tr>
<td>PEI-coated GCE</td>
<td>Potentiometric Flow injection measurement</td>
<td>Covalent</td>
<td>1.0</td>
<td>NR</td>
<td>Dichlorvos</td>
<td>10</td>
<td>NR</td>
<td>[65]</td>
</tr>
<tr>
<td>PEI/SPE</td>
<td>Amperometric</td>
<td>Noncovalent Adsorption</td>
<td>1.0 x 10^-4 0.48 x 10^-1 0.61 x 10^-3</td>
<td>NR</td>
<td>Dichlorvos DiazinonFenthion</td>
<td>2 days</td>
<td>NR</td>
<td>[41]</td>
</tr>
<tr>
<td>PANI/CNT wrapped with ssDNA/Au electrode</td>
<td>Electrochemical</td>
<td>Enzyme</td>
<td>7.5 x 10^-3</td>
<td>0.018–0.45 and 1.0 x 10^-5 and 1.0</td>
<td>Methyl parathion and chlorpyrifos</td>
<td>15</td>
<td>5</td>
<td>[67]</td>
</tr>
<tr>
<td>AuNPs–PPy nanowires composite film modified GCE</td>
<td>Electrochemical</td>
<td>Enzyme</td>
<td>3.02 x 10^-3</td>
<td>1.89–17.0 0.030–1.51 and 3.027–75.67</td>
<td>Methyl parathion</td>
<td>12</td>
<td>30</td>
<td>[68]</td>
</tr>
<tr>
<td>PPy and PANI copolymer doped with MWCNTs/GCE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>0.1 x 10^-3 and 1.5 x 10^-3</td>
<td>0.1–50 x 10^-3 and 1.5–40 x 10^-3</td>
<td>Malathion and chlorpyrifos</td>
<td>10</td>
<td>60</td>
<td>[70]</td>
</tr>
</tbody>
</table>

Note: NR, not reported.

Al₂O₃ sol–gel matrix [77], sol–gel matrix on 7,7,8,8-tetracyanoquinodimethane (TCNQ) [78], AuNP–SiSG [79], alumina sol–gel [80], bromothymol blue doped sol–gel film [81], zinc oxide sol–gel [82], and silica sol–gel film [83].

SPE-based AChE biosensors

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

Supports used for immobilization of enzyme: TMOS sol–gel film/ SPE [40], Al₂O₃ sol–gel matrix SPE [77], sol–gel matrix on TCNQ
Quantum dot-based AChE biosensors

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

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

Table 7 provides a comparative analysis of properties of QD-based AChE biosensors.

Nanoparticle-based AChE biosensors

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

Supports used for immobilization of enzyme: AuNPs–CaCO3 bioconjugate/Au electrode [94], Fe3O4NP/MWCNTs/Au electrode [95], Fe3O4NP/MWCNTs/indium tin oxide (ITO) electrode [96], AuNPs/PB/GCE [97], MWCNTs–gold nanocomposites/GCE [98], ZrO2/CHIT composite film/GCE [99], gold–platinum bimetallic NPs/GCE [45], AuNPs/GCE [100], AuNPs–MWCNTs/GCE [101], Pb and CHIT/GCE [43], TiO2–decorated graphene/GCE [102], graphite, nanoplatelet–CHIT composite/GCE [103], calcium carbonate–CHIT composite film/GCE [104], Cds–decorated graphene nanocomposite [105], CHIT–GNPs/Au electrode [106], MWCNTs–CHIT composite/GCE [42], AuNPs/gold electrode [107], MWCNTs/AuNPs–CHIT/GCE [18], PbO2/TiO2/Ti [108], and Pb–CHIT/GCE [109].

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

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

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

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

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol–gel crystals derived from TMOS</td>
<td>Optical</td>
<td>Encapsulation</td>
<td>0.94</td>
<td>42.19</td>
<td>NaledMecarbam</td>
<td>5</td>
<td>30</td>
<td>[73]</td>
</tr>
<tr>
<td>Sol–gel film on a glass cap</td>
<td>Fiber-optic</td>
<td>Amperometric</td>
<td>0.098</td>
<td>0.024, 0.015, and 0.012</td>
<td>Paraaxon, dichlorvos, and chlorpyrifos-ethyl oxon</td>
<td>30</td>
<td>NR</td>
<td>[74]</td>
</tr>
<tr>
<td>Silica sol–gel</td>
<td>Amperometric</td>
<td>Encapsulation</td>
<td>1.0 × 10⁻³</td>
<td>0.008–0.81, 1.0 and 3.0 × 10⁻¹</td>
<td>Oxidemeton methyl dichlorvos</td>
<td>20</td>
<td>21</td>
<td>NR</td>
</tr>
<tr>
<td>TMOS sol–gel–GCE</td>
<td>Amperometric</td>
<td>Encapsulation</td>
<td>2.26</td>
<td>2.26–31.67</td>
<td>Dichlorvos</td>
<td>15</td>
<td>NR</td>
<td>[76]</td>
</tr>
<tr>
<td>Chromoionophore (ETH5294) doped sol–gel film</td>
<td>Optical fiber</td>
<td>Encapsulation</td>
<td>3.0 × 10⁻⁴</td>
<td>0.127–5.010</td>
<td>Methyl parathion and acephate</td>
<td>20 and 4</td>
<td>30</td>
<td>[83]</td>
</tr>
<tr>
<td>AuNPs–SiSG/GCE</td>
<td>Electrochemical</td>
<td>Hydrogen bonds</td>
<td>0.44</td>
<td>NR</td>
<td>Monocrotophos</td>
<td>10</td>
<td>30</td>
<td>[79]</td>
</tr>
<tr>
<td>Alumina sol–gel–sonogel–carbon electrode</td>
<td>Amperometric</td>
<td>Encapsulation</td>
<td>2.5 × 10⁻⁴</td>
<td>0.5</td>
<td>Chlorpyriphos-ethyl oxon</td>
<td>10</td>
<td>50</td>
<td>[80]</td>
</tr>
<tr>
<td>Bromethylom blue doped sol–gel film</td>
<td>Optical fiber</td>
<td>Encapsulation</td>
<td>0.11</td>
<td>0.14–5.70</td>
<td>Chlorpyrifos</td>
<td>8</td>
<td>60</td>
<td>[81]</td>
</tr>
<tr>
<td>Zinc oxide sol–gel/SPE</td>
<td>Amperometric</td>
<td>Electrostatic interactions</td>
<td>3.0 × 10⁻¹</td>
<td>3.7 × 10⁻¹ and 4.7</td>
<td>NaledMecarbam</td>
<td>5</td>
<td>30</td>
<td>[73]</td>
</tr>
<tr>
<td>Silicasol–gel film/carbon paste electrode</td>
<td>Amperometric</td>
<td>Encapsulation</td>
<td>0.47</td>
<td>0.27–4.09</td>
<td>NaledMecarbam</td>
<td>5</td>
<td>30</td>
<td>[73]</td>
</tr>
</tbody>
</table>

Note: NR, not reported.
their performance and operational characteristics. Most AChE immobilization chemistry used to attach the enzyme determine the basic design of these devices, the electrode material, and the food matrices. AChE biosensors also show promise in public safety. The majority of AChE biosensors are designed to detect AChE inhibition-based biosensors / C.S. Pundir, N. Chauhan / Anal. Biochem. 429 (2012) 19–31

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

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃ sol–gel matrix SPE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>0.01</td>
<td>0.1–80</td>
<td>Chlorpyrifos-ethyl oxon</td>
<td>10</td>
<td>5</td>
<td>[77]</td>
</tr>
<tr>
<td>Sol–gel matrix on TCNQ modified SPE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>1 × 10⁻²</td>
<td>7.2 × 10⁻³</td>
<td>1.6 × 10⁻¹</td>
<td>10</td>
<td>3</td>
<td>[88]</td>
</tr>
<tr>
<td>SPE (TCNQ mediator in the graphite electrode)</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>0.126</td>
<td>0.063–0.315</td>
<td>Carbaryl, carbofuran, and parathion</td>
<td>15</td>
<td>92</td>
<td>[85]</td>
</tr>
<tr>
<td>PB modified SPE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>1.0 × 10⁻⁴</td>
<td>1.0 × 10⁻¹</td>
<td>Aldicarb, carbaryl, paraoxon, and chlorpyrifos-methyl oxon</td>
<td>15</td>
<td>92</td>
<td>[84]</td>
</tr>
<tr>
<td>CoPC/SPCEs o-Phenylenediamine onto carbon/CoPC SPE</td>
<td>Amperometric</td>
<td>Crosslinking</td>
<td>4.9 × 10⁻⁴</td>
<td>1.0 × 10⁻¹</td>
<td>Carbofuran, parathion, and azinphos</td>
<td>15</td>
<td>NR</td>
<td>[87]</td>
</tr>
<tr>
<td>Graphite–epoxy composite/SPE</td>
<td>Amperometric</td>
<td>Crosslinking</td>
<td>10.0 × 10⁻⁴</td>
<td>1.0 × 10⁻¹</td>
<td>Paraoxon and carbofuran</td>
<td>15</td>
<td>5</td>
<td>[86]</td>
</tr>
<tr>
<td>SPE</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>0.18</td>
<td>0.18–54.00</td>
<td>Paraoxon</td>
<td>10</td>
<td>NR</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and</td>
<td>0.01</td>
<td>0.018–0.181</td>
<td>Paraoxon and malaoxon</td>
<td>15</td>
<td>3</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and</td>
<td>6.3 × 10⁻⁴</td>
<td>6.3 × 10⁻⁴</td>
<td>Pirimicard</td>
<td>10</td>
<td>92</td>
<td>[86]</td>
</tr>
</tbody>
</table>

Note. NR, not reported.

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

<table>
<thead>
<tr>
<th>Electrode material/Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdTe QDs/AuNPs/CHIT/GCE</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>1.34</td>
<td>4.4 × 10⁻³</td>
<td>Monocrotophos</td>
<td>8</td>
<td>30</td>
<td>[92]</td>
</tr>
<tr>
<td>CdTe QDs/Au electrode</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>2.98 × 10⁻³</td>
<td>4.96 × 10⁻⁴</td>
<td>Carbofuran</td>
<td>10</td>
<td>30</td>
<td>[106]</td>
</tr>
<tr>
<td>Poly(allylamine hydrochloride)/CdTe QDs/glass</td>
<td>Optical</td>
<td>Electrostatic interaction</td>
<td>1.05 × 10⁻⁵</td>
<td>1.0 × 10⁻⁶</td>
<td>Carbofuran, parathion</td>
<td>15</td>
<td>35</td>
<td>[93]</td>
</tr>
</tbody>
</table>

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

Applications of AChE biosensors

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

Summary and conclusion

AChE biosensors are strong candidates for screening pesticide residues and are becoming more and more relevant in environmental and food analysis. Compared with traditional chromatography...
### Table 8
AChE biosensors based on nanoparticles for pesticide detection.

<table>
<thead>
<tr>
<th>Electrode material/ Immobilization matrix</th>
<th>Type of transducer/technique</th>
<th>Immobilization method</th>
<th>Detection limit (µM)</th>
<th>Linearity (µM)</th>
<th>Application/Analyte/Inhibitors</th>
<th>Incubation time (min)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs–CaCO₃ bioconjugate/ Au electrode</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>$0.1 \times 10^{-3}$</td>
<td>$0.1 \times 10^{-3}$</td>
<td>Malathion and chlorpyrifos</td>
<td>10</td>
<td>90</td>
<td>[70]</td>
</tr>
<tr>
<td>Fe₃O₄NP/MWCNTs/Au electrode</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>$0.1 \times 10^{-3}$</td>
<td>$0.1-10 \times 10^{-3}$</td>
<td>Malathion, chlorpyrifos, monocrotophos, and endosulfan</td>
<td>10</td>
<td>60</td>
<td>[95]</td>
</tr>
<tr>
<td>Fe₃O₄NP/MWCNTs/ITO electrode</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>$0.1 \times 10^{-3}$</td>
<td>$0.1-10 \times 10^{-3}$</td>
<td>Malathion, chlorpyrifos, monocrotophos, and endosulfan</td>
<td>10</td>
<td>90</td>
<td>[96]</td>
</tr>
<tr>
<td>AuNPs/PB/GCE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>$7.0 \times 10^{-3}$</td>
<td>$28 \times 10^{-3}$</td>
<td>Methamidophos</td>
<td>10</td>
<td>7 days</td>
<td>[100]</td>
</tr>
<tr>
<td>AuNPs–MWCNTs/GCE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>$7.0 \times 10^{-3}$</td>
<td>$170 \times 10^{-3}$</td>
<td>Methamidophos</td>
<td>30</td>
<td>NR</td>
<td>[101]</td>
</tr>
<tr>
<td>PB and CHIT/GCE</td>
<td>Amperometric</td>
<td>Glutaraldehyde crosslinking</td>
<td>$0.113 \times 10^{-4}$</td>
<td>$0.45 \times 10^{-4}$</td>
<td>Dichlorvos, omethoate, trichlorfon, and phoxim</td>
<td>10</td>
<td>NR</td>
<td>[43]</td>
</tr>
<tr>
<td>TiO₂-decorated graphene/ GCE</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>$1.4 \times 10^{-3}$</td>
<td>$4.9-74.5$</td>
<td>Carbaryl</td>
<td>3</td>
<td>20</td>
<td>[102]</td>
</tr>
<tr>
<td>Graphitenanoplatelet–CHIT composite/GCE</td>
<td>Voltammetric</td>
<td>Covalent</td>
<td>$1.58 \times 10^{-4}$</td>
<td>$74.5-9.9 \times 10^{-4}$</td>
<td>Chloropyrifos</td>
<td>10</td>
<td>10</td>
<td>[103]</td>
</tr>
<tr>
<td>Calciumcarbonate–CHIT composite film/GCE</td>
<td>Electrochemical</td>
<td>Entrapment</td>
<td>$3.7 \times 10^{-3}$</td>
<td>$0.018-0.759$</td>
<td>Methyl parathion</td>
<td>10</td>
<td>NR</td>
<td>[104]</td>
</tr>
<tr>
<td>CdS-decorated graphene nanocomposite CHIT–GMP/Au electrode</td>
<td>Amperometric</td>
<td>Adsorption</td>
<td>$3.4 \times 10^{-3}$</td>
<td>$2.84-14.24$</td>
<td>Carbaryl</td>
<td>2</td>
<td>20</td>
<td>[105]</td>
</tr>
<tr>
<td>MWCNTs–CHIT composite/ GCE</td>
<td>Amperometric</td>
<td>Covalent</td>
<td>NR</td>
<td>0.3</td>
<td>Malathion</td>
<td>15</td>
<td>NR</td>
<td>[106]</td>
</tr>
</tbody>
</table>

**References:** [70], [95], [96], [70], [97], [98], [99], [45], [100], [101], [43], [102], [103], [104], [105], [106], [42]
and other methods, the strengths of AChE biosensors are that they are very selective, sensitive, and disposable and also work with complete automation and provide rapid results.

Attempts have been made to summarize the salient features of various acetylcholine biosensors reported so far. Most of the reports have focused on the use of AChE for detection of specifically organophosphate and carbamate pesticides. AChE sensors have the potential to be a billion-dollar market, and the technology needs improvement in biological stability, signal transduction, precision, and cost-effectiveness. The role of matrices for biosensing and the characteristics of various biosensors, in terms of response time, detection limit, and linear range, have been delineated. It is suggested that microfabrication technology and nanotechnology as a patterning method and biomolecules as templates for the simultaneous detection of clinically important metabolites and rapid screening of pesticides is still needed. The use of biomolecules to grow NPs has great promise in the future of biosensing and design of bioelectronic systems. We believe that the use of biocatalytic nanostructure growth, using dip pen nanolithography as a patterning method and biomolecules as templates for nanostructure synthesis, holds great promise in future nanotechnologies.

**Future perspectives**

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

**References**


