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REVIEW ARTICLE

Nanomaterials in electrochemical biosensors for pesticide detection: advances and challenges in food analysis

Fabiana Arduini[1,2] · **Stefano Cinti**[1] · **Viviana Scognamiglio**[3] · **Danila Moscone**[1,2]

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Abstract This overview (with 114 refs.) covers the progress made between 2010 and 2015 in the field of nanomaterial based electrochemical biosensors for pesticides in food. Its main focus is on strategies to analyze real samples. The review first gives a short introduction into the most often used biorecognition elements. These include (a) enzymes (resulting in inhibition-based and direct catalytic biosensors), (b) antibodies (resulting in immunosensors), and (c) aptamers (resulting in aptasensors). The next main section covers the various kinds of nanomaterials for use in biosensors and includes carbonaceous species (carbon nanotubes, graphene, carbon black and others), and non-carbonaceous species in the form of nanoparticles, rods, or porous materials. Aspects of sample treatment and real sample analysis are treated next before discussing vanguard technologies in tailor-made food analysis.

Keywords Enzymatic biosensor · Immunosensor · Aptasensor · Carbon nanotubes · Nanorods · Nanoparticles · Graphene · Carbon black · Sustainable food chain · Food safety

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✉ Fabiana Arduini
fabiana.arduini@uniroma2.it

[1] Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Via della Ricerca Scientifica, 00133 Rome, Italy

[2] Consorzio Interuniversitario Biostrutture e Biosistemi "INBB", Viale Medaglie d'Or, 305, 00136 Rome, Italy

[3] Dipartimento di Scienze Bio-Agroalimentari, Istituto di Cristallografia (IC-CNR), Via Salaria km 29.300, 00015 Monterotondo, Rome, Italy

Introduction

The scientific community working in food science is nowadays continuously demanded to give adequate answers to the consumers, whose perception about food safety and quality is constantly increasing. Besides, there is an overall tendency to connect food and health with the main purpose of boosting wellbeing and preventing future diseases [1].

Several concerns regarding the increasing of the global population, the intensive agriculture and animal farming, as well as food handling, processing, and distribution, pose noticeable challenges to the food sector, negatively affecting food safety and consumer health. A crucial issue regards food contamination by pesticides, which are nowadays an integral part of agriculture [2]. Indeed, the last report of U.S. Environmental Protection Agency (EPA) released on February 2011 highlighted that the pesticide use in the United States was estimated at 1.1 billion pounds in 2007 and the use of conventional pesticides decreased about 3 % from 2002 to 2007 and 11 % from 1997 to 2007; however, approximately 857 million pounds of conventional active pesticides were still applied in 2007. In the case of organophosphate insecticides, their use decreased about 44 % from 2002 to 2007, 63 % from 2000 to 2007, and 55 % from 1997 to 2007; however, about 33 million pounds of organophosphate insecticides were still applied in 2007 [3].

The negative impact of these compounds on the environment was demonstrated by listing pesticides as priority hazardous substances in the field of European Water Policy, such as organophosphorus chlorfenvinphos [4]. Because of their adverse health effects, most countries have established maximum

residue levels (MRL) in food and animal feed [5]. Recently the European Commission has reported the guidelines for the 2050 food system, which comprise the development of effective integrated approaches to establish, promote, and support a sustainable food chain including measures to ensure integrity in terms of food safety and quality [6]. To this regard, biosensor technology is significantly establishing, as reflected by the wide literature in the field, and represents an edge over the other methods, thanks to its inexhaustible development opportunity and the huge market potential [7, 8]. This technology provides sophisticated and tailored analytical systems with high sensitivity, specificity, reliability, and speed of analysis, along with low costs, miniaturization, and portability.

Undeniably, last trends on material science, nanotechnology, and biomimetic design provided a significant enhancement of the biosensor performances for their applications in food and related sectors [9]. In particular, new smart sensing devices have been designed in the last years by taking advantage of the recognized effects arising from the reduced sizes of nanomaterials. Numerous types of nanomaterials have been employed for biosensor development, from spheres and particles (metal nanoparticles, magnetic nanobeads, quantum dots), to nanotubes, nanowires, nanorods, nanofibers, as well as nanocomposites, nanofilms, nanopolymers, and nanoplates. These materials are able to enhance the performances of detection systems, thanks to their unique physical, chemical, mechanical, magnetic, and optical properties, such as strong absorption band in the visible region, high electrical conductivity, and good mechanical features. These special features help to tune level of sensitivity as required by the analysis, and allow different detection limits depending from the matrix to be analysed. In addition, the use of nanomaterials to functionalize the bioelements can improve their stability and specificity, yielding also reproducibility and reliability. Furthermore, nanotechnology allows the miniaturization and the integration of biocomponents, transduction systems, electronics, and microfluidics in sophisticated architectures, able to perform high throughput analyses as lab-on-chip devices for rapid and low cost screening using small food samples [10–16].

Furthermore, nanomaterials can provide important benefit in the construction of biosensors when in combination with electrochemical transductors, due to their electrocatalytic activity and high surface area. The first property allows for a fast and sensitive measurement at low applied potential, avoiding electrochemical interferences. The latter one consents a higher loading of the biocomponents, enhancing the sensitivity of the measurement.

The use of electrochemical transduction is likewise promising at the same strength of nanotechnology, posing additional advantages in comparison with other transduction systems (e.g. optical), such as the capability to operate in turbid media exploiting easy to use and cost effective equipments. In addition, in-situ and/or on-line measurements are allowed with high sensitivity by different electroanalytical techniques, including square wave voltammetry, chronopotentiometry, chronoamperometry, and differential pulse voltammetry. For these reasons, electrochemical biosensors are the most used in measuring pesticides [17–21].

In this review, progresses in the development of biosensors for pesticide monitoring in food are described, focusing specifically on electrochemical biosensors based on nanomaterials developed over the last 5 years. The following sections provide an extensive overview on:

i) The different bioelements that, coupled with nanomaterials, are usually employed for electrochemical sensing of pesticide in food;

ii) The innovative and emerging nanomaterials tailored for a smart biosensor configuration.

In addition, sample treatment is also debated taking into consideration the importance of the procedures to obtain treated samples suitable for fast and accurate analysis. Finally, a section has been dedicated to the vanguard technologies that help researchers to envisage alternative strategies for the development of tailor-made electrochemical biosensors for food analysis.

Bioelements

Enzymatic biosensor

Enzymatic biosensors are based on enzymes in close contact with the transducer, selectively reacting with their substrate. As depicted in Fig. 1a, enzymatic biosensors represent the most attractive area of research in biosensing field for food analysis of the last 5 years, thanks to their robustness, easiness of immobilization, and fast response when compared to antibody based biosensors.

These biosensors are able to detect the target analyte by means of: i) an inhibition mechanism, in which the pesticide inhibits the enzymatic activity, thus decreasing its response; ii) a catalytic mechanism, where the pesticide represents the direct enzymatic substrate and the enzymatic product is measured.

Inhibition-based biosensors

Among inhibition-based biosensors, cholinesterase enzymes are the most exploited (Fig. 1b) for organophosphorous and carbamic pesticides, which act as irreversible inhibitors able to phosphorylate the serine residue in the active site of the enzyme.

This inhibition mechanism permits the quantification of the pesticide content by measuring the enzyme activity in the absence and in the presence of the analyte. The procedure encompasses the following steps: i) measurement of the enzymatic activity, ii) incubation in a sample (eventually

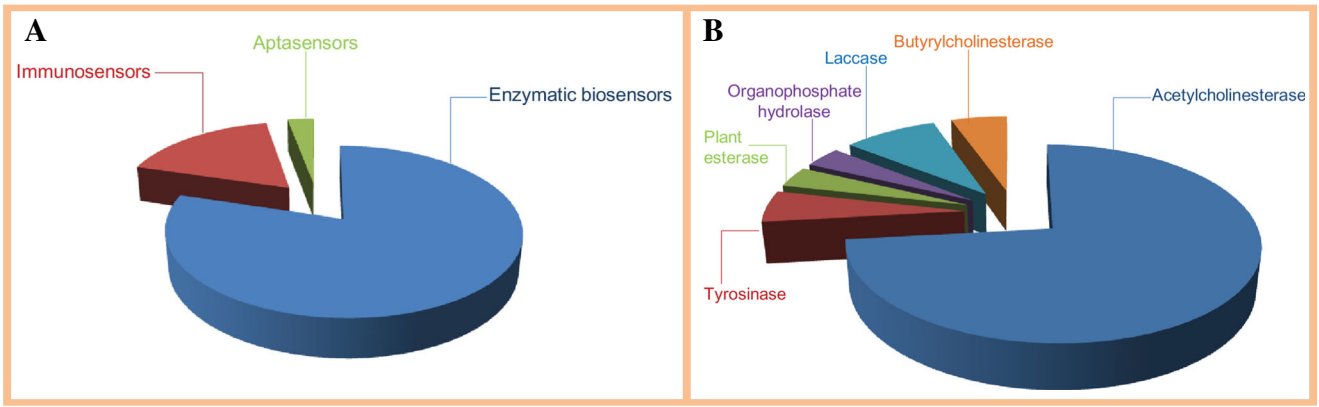

Fig. 1 **a** Distributions of the biosensors and **b** the enzymes employed in the biosensor construction (data taken on January 2016 from www.googlescholar.com using the keywords: electrochemical, biosensor, pesticide, food)

contaminated with pesticides), and iii) measurement of the inhibited enzymatic activity.

The amount of carbamic and organophosphorus pesticides is quantified by using the following Eq. (1):

$$I\% = ((I_0–I_1)/I_0) \times 100 \quad (1)$$

where I% is the degree of inhibition, I_0 is the enzyme activity *before* the exposure to the sample and I_1 is the enzyme activity *after* the exposure to the sample. In the presence of pesticides, a decrease of the enzymatic activity is observed, highlighted by the decrease of I% value.

Several electrochemical biosensors have been described in literature based on acetylcholinesterase (AChE) enzyme [21–24]. AChE is able to hydrolyze acetylcholine following the Eq. (2):

$$\text{Acetylcholine} + H_2O \rightarrow \text{Choline} + \text{Acetic acid} \quad (2)$$

This reaction can be monitored measuring i) the variation of pH by potentiometric transduction [25]; ii) the variation of conductibility by conductometric transduction [26]; iii) the detection of hydrogen peroxide by amperometric transduction, using a bi-enzymatic approach (in combination with choline oxidase enzyme) [27]. The latter approach involves the deployment of bare platinum electrodes [28], carbon based electrodes modified with the electrochemical mediators [29, 30], or nanomaterials [31, 32] where the produced hydrogen peroxide is then oxidized or reduced, respectively.

To avoid the use of a multi-enzyme approach, with the drawback of enhancing the complexity of the measurement, the non-natural substrate acetylthiocholine was then exploited for the development of mono-enzymatic amperometric biosensors, since the enzymatic product thiocholine is electroactive. However, thiocholine determination using bare electrodes is prohibitive, owing to the high potential required and to fouling problems affecting the thiol detection [33]. The use of sensors modified with nanomaterials has increased the development of this type of biosensors, because of the electrocatalytic properties of several nanomaterials that accomplish the thiocholine detection at low applied potential, overcoming the fouling problem. To this regard, Liu et al. [34] reported on the utilization of the hybrid multi-walled carbon nanotubes (MWCNT)/graphene oxide nanoribbons to assembly an AChE biosensor for carbaryl detection (Fig. 2a). The catalytic activity of MWCNT/graphene oxide nanoribbon film was better to that of just MWCNTs. MWCNT/graphene oxide nanoribbon film was able to oxidize thiocholine at around 0.7 V (vs. saturated calomel electrode, SCE). The resulted biosensor exhibited satisfactory performances towards carbaryl detection, characterized by a wide linear range from 5 to 5000 nM and a limit of detection equal to 1.7 nM. The practicality of the proposed method was demonstrated by the recovery test by adding different amounts of carbaryl into cabbage samples. The recoveries were from 95.5 % to 96.8 %, indicating that the proposed method can be used for direct analysis of real samples.

A pesticide biosensor based on AChE immobilized in a core–shell structure of CNTs and on polyaniline for detection of methomyl and carbaryl in liquefied apple, cabbage and broccoli samples was developed by Cesarino et al. [35]. The use of polyaniline is motivated by the fact that the electrochemical formation of polymer layers offers significant advantages, such as complete coverage of the active surface, greater control over film thickness, and enhanced reproducibility. The square wave voltammetry carried out using a glassy carbon electrode modified with MWCNTs and polyaniline showed a well-defined oxidation peak at +0.025 V vs. Ag/AgCl ascribable to the thiocholine oxidation, demonstrating the low applied potential required for thiocholine detection. This biosensor is characterized by a detection limit of 0.28 mg kg^{-1} (1.4 μM) for carbaryl and 0.15 mg kg^{-1} (0.95 μM) for methomyl. As highlighted by the authors, the detection limits achieved are adequate to

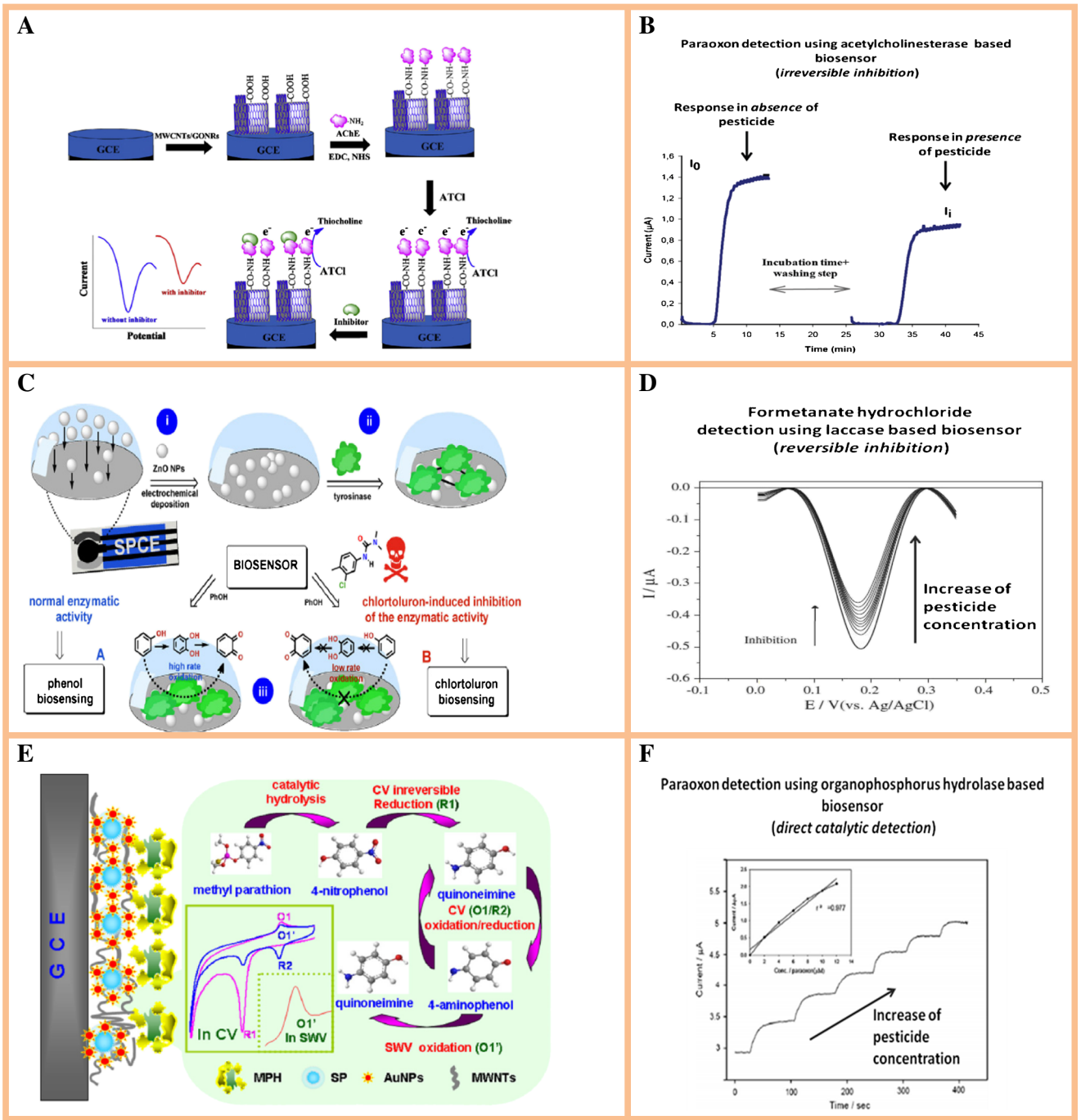

Fig. 2 Examples of enzymatic based biosensors. **a** Schematic illustration of the stepwise AChE biosensor fabrication process [Reprinted with permission from reference 34]; **b** Typical response of paraoxon detection using BChE based biosensor embedded in a flow system [Reprinted with some modifications with permission from reference 40]; **c** Schematic representation of (i) electrochemical deposition of ZnO NPs using cyclic voltammetry (ii) functionalization of ZnO/SPCE electrode using tyrosinase/glutaraldehyde, iii) (A) detection of phenol and (B)inhibition-based detection of chlortoluron [Reprinted with permission from reference 44]; **d** A typical response using laccase based biosensor [Reprinted with some modifications with permission from reference 48]; **e** Schematic illustration of the methyl parathion based biosensor mechanism [Reprinted with permission from reference 50] and **f** the typical response [Reprinted with some modifications with permission from reference 49]

monitor contaminations with such pesticides according to the National Health Surveillance Agency (ANVISA) in Brazil, which established a MRL of 3.0 mg kg^{-1} for methomyl in cabbage and broccoli and 2.0 mg kg^{-1} for carbaryl in apples (Agência Nacional de Vigilância Sanitária). Since AChE biosensor is able to detect both classes of organophosphates and carbamates, the successive analysis with High Performance Liquid Chromatography (HPLC) was necessary to identify

the nature of the pesticide. Thus, the authors performed a comparative study by using both HPLC and biosensor approach to detect carbaryl in apple samples, and methomyl in cabbage and broccoli samples. Any significant differences between these methods were underlined, demonstrating at 95 % confidence level the accuracy and reliability of the implemented biosensor [35].

Together with carbon based and metallic based nanomaterials, nanosized electrochemical mediators were also employed to design AChE based biosensors to allow the detection of thiocholine at low potential. For instance, a Prussian Blue nanocube/reduced graphene oxide nanocomposite was exploited for the development of a novel AChE biosensor. This architecture allowed the thiocholine detection with an over-potential decrease of 460 mV vs. Ag/AgCl compared to the bare electrodes, due to the electrocatalytic activity of Prussian Blue nanocubes towards the oxidation of thiocholine. The AChE biosensor showed rapid response and high sensitivity for the detection of monocrotophos with a linear range from 1.0 to 600 ng mL^{-1} (4.5×10^{-9} - 2.7×10^{-6} M) and a detection limit of 0.1 ng mL^{-1} (4.5×10^{-10} M). Suitability in real samples was also demonstrated, testing this biosensor in cucumber solutions with recovery values of 97–104 %. The biosensor was stored at 4 °C in dry conditions showing 80 % of initial current response after a 30-day storage period [36].

In addition to AChE extracted from different organisms, including *electric eel* [36–38], *Drosophila melanogaster* [39], and *bovine erythrocyte* [35], butyrylcholinesterase (BChE) enzyme from *horse serum* has been also exploited for biosensor development, using butyrylthiocholine as substrate.

As an example, BChE was used as biocomponent to be immobilized on a screen-printed electrode (SPE) modified with Prussian Blue nanoparticles. The detection of paraoxon was carried out evaluating its inhibitory effect on BChE, by measuring the produced thiocholine at a working voltage of +200 mV vs. Ag/AgCl, thanks to the electrocatalytic properties of Prussian Blue nanoparticles. This biosensor was embedded in a flow system, and reached a detection limit of 1 μg L^{-1} (3.6×10^{-9} M) of paraoxon. A typical response was reported in Fig. 2b showing the measurement before and after the exposure to paraoxon. In this case, the biosensor was exposed for 10 min (incubation time) to the contaminated sample. The analytical system was then challenged in drinking water samples, obtaining a recovery value of 90 ± 5 %. The stability in non-operational conditions (storage stability at room temperature in dry conditions) was also investigated up to 60 days in flow system toward the substrate solution, giving responses almost constantly after eight weeks of investigation, and confirming the robustness of the immobilization procedure. This feature, together with repeatability, is certainly fundamental in the construction of a biosensor with market attractiveness [40].

As depicted by the above reported results that reflect the overall trend in literature, the BChE seems to be characterized by higher storage stability, which is an important added value for the future commercialization in the agrifood biosensor market [41]. Only recently, a number of cholinesterase enzymes have been extracted from vegetable sources, avoiding animal use. For instance, AChE was purified from maize seedlings and immobilized on iron oxide nanoparticles and on a carboxylated MWCNT modified Au electrode. Also in this case, the authors underlined the synergic action of nanomaterials employed, namely iron oxide nanoparticles and carboxylated MWCNTs that accomplished the thiocholine detection at a low potential (+0.4 V vs. Ag/AgCl). Using this biosensor, several pesticides were tested like malathion, chlorpyrifos, monocrotophos, and endosulfan, with a detection limit of 0.1×10^{-9} M for malathion and chlorpyrifos, 1×10^{-9} M for monocrotophos, and 10×10^{-9} M for endosulfan. This biosensor demonstrated stability over 2-months, when stored dry at 4 °C. Furthermore, the suitability of this biosensor for pesticide detection in tap water and milk was assessed by recovery studies. In the case of tap water, recovery studies were carried out using all pesticides and a precision smaller than 14 % and accuracy within 98–110 % were achieved. In the case of milk samples, malathion was taken as a pesticide model and recovery values within 107 and 109 % were obtained [42].

An esterase enzyme extracted by plants was also exploited for the design of a biosensor where the esterase was immobilized on a chitosan/gold nanoparticle-graphene nanosheet composite. This biosensor measured 50 ng L^{-1} (0.19×10^{-9} M) of methyl parathion and 0.5 μg L^{-1} (1.51×10^{-9} M) of malathion (S/*N* = 3). The good performances achieved, as highlighted by the authors, might be attributed to the concurring effects of several matters: i) the esterase can be strongly inhibited by pesticides; ii) gold nanoparticle-graphene nanosheet increased the probe surface area allowing a monolayer immobilization of the enzyme, also enhancing the electron transfer, and iii) chitosan provided a favorable microenvironment for the enzyme. In addition, the advantage of the esterase from plant sources relies also on its large scale purification at a significant lower cost compared to AChE from animal sources [43].

Another type of inhibition based biosensors relies on the use of tyrosinase and laccase enzymes. In this case the inhibition is of a reversible nature, and the protocol encompasses only two steps: i) measurement of the enzymatic activity in the absence of pesticides, and ii) measurement of the enzymatic activity in the presence of pesticides.

The amount of pesticide is quantified by using the Eq. (1) reported above, where I_0 is the enzyme activity in the *absence* of pesticide and I_1 is the enzyme activity in the *presence* of pesticide.

For instance, a biosensor based on tyrosinase inhibition for chlortoluron, a widely used herbicide in cereals, was reported [44].The authors projected a sensing platform using tyrosinase immobilized onto SPEs previously modified with ZnO nanoparticles (Fig. 2c). This biosensor was able to selectively detect phenol at an applied potential of −0.2 V vs. Ag/AgCl, and reaching a limit of detection equal to 0.02×10^{-6} M. In the presence of chlortoluron, the enzymatic activity was inhibited depending on the herbicide concentration, and a low detection limit equal to 0.47×10^{-9} M was achieved. In case of a reversible inhibition, the medium exchange method [45] is not applicable, since the interaction between the enzyme and inhibitors does not rely in a covalent bonding; thus the interference should be deeply investigated. In this case, the biosensor was tested against interferences like aniline (50×10^{-6} M), ascorbic acid (50×10^{-6} M), pyrogallol (50×10^{-6} M), and copper (5×10^{-6} M), and only copper sulfate produced a small variation in the steady-state current, since a binuclear copper complex is contained in a characteristic tyrosinase active site involved in the oxidation of phenol. Tap water samples spiked with 50×10^{-9} M of chlortoluron gave recovery percentages of 98 ± 2.6 %, demonstrating the suitability of this biosensor in real sample analysis.

Tyrosinase based biosensor was also exploited for atrazine detection. Tortolini et al. developed this type of biosensor using bare SPE as well as SPE modified with graphene or MWCNTs. In the presence of catechol as substrate, atrazine can be determined thanks to its inhibitory activity towards the enzyme, which catalyses the oxidation of catechol to o-quinone. Under optimum experimental conditions, the best performance in terms of catalytic efficiency was demonstrated by using MWCNTs-modified SPEs. This inhibition biosensor reached a detection limit of 0.3 mg L^{-1} (1.4×10^{-6} M) [46].

Laccase was also employed for the determination of pesticides [47, 48]. Formetanate hydrochloride was measured using biosensor based on laccase inhibition and gold electrode modified with gold nanoparticles. In this case, gold nanoparticles were used as nanomaterial and aminophenol as substrate. This biosensor was successfully applied to formetanate hydrochloride determination. A typical response was reported in Fig. 2d showing a rapid response without requiring an incubation time. The limit of detection of 9.5×10^{-8} M (0.02×10^{-4} mg/kg on a fresh fruit weight basis) was achieved. Recovery studies in mango and grapes using five spiking levels gave recovery values in the range from 95.5 ± 2.9 (grapes) to 108.6 ± 2.5 % (mango) [48].

Direct catalytic biosensors

Biosensors based on the use of organophosphorus hydrolase have been also extensively reported to detect organophosphates as direct substrates, providing responses *directly* proportional to the pesticide amount (Fig. 2f) [49]. For this reason, organophosphorus hydrolase is well suited to perform continuous monitoring of the analyte concentration, even if the sensitivity is usually lower than AChE based biosensors. The principle relies on the hydrolysis of *p*-nitrophenyl group of the target analyte (e.g. paraoxon, parathion) to *p*-nitrophenol, which is electrochemically detected. As an example, a biosensor based on methyl parathion hydrolase and electrode modified with gold nanoparticles synthesized on silica particles and MWCNTs was reported in literature (Fig. 2e) [50]. This biosensor was capable to detect methyl parathion with a detection limit of 0.3 ng mL^{-1} (1×10^{-9} M), showing satisfactory selectivity against methyl parathion analogs, namely ethyl parathion and paraoxon. The recovery test with known amounts of methyl parathion in garlic samples resulted from 95.0 % to 102.3 %, demonstrating the good accuracy of this biosensor.

Immunosensors

Immunoassays are biochemical tests able to quantify the concentration of an analyte in a sample through the highly specific molecular recognition between an antibody and the corresponding epitope of an antigen (target analyte), producing a measurable signal in response to the binding event. Commonly, immunoassays require the use of enzymes as label of the formed immunocomplex. The requirement for faster analyses has moved the research activities to replace enzyme labels with nanomaterials as well as to avoid labels through the development of label free immunosensors [51].

An example of immunosensor based on quantum dots as label in place of enzymes has been reported by Valera et al. [52]. In this case, the use of quantum dots allowed a fast detection of the immunocomplex, avoiding the time of reaction between the enzyme (used ad immunocomplex label) and its substrate. In detail, an immunosensor for the pesticide paraquat was assembled using paraquat specific antibodies labelled with CdS nanoparticles and the antigen biofunctionalized with magnetic μparticles confined on the working electrode surface. The formation of the immunocomplex was quantified by measuring the metal ions released in an acid solution from CdS nanoparticles (Fig. 3a). Due to the amplification effect produced by CdS nanoparticles on the electrochemical signal, a very high detectability was reached; indeed paraquat was detected with an IC50 equal to 0.18 ± 0.31 μg L^{-1} (7×10^{-10} M). This novel immunosensor was successfully applied for paraquat detection in potatoes with a detection limit equal to 1.4 μg kg^{-1}, far below the maximum residue limit (MRL, 20 μg kg^{-1}) established by the EU for this pesticide.

In the case of label free immunosensors, novel displacement immunoassays were proposed based on the use of analogues in place of labeled analytes to competitively bind the

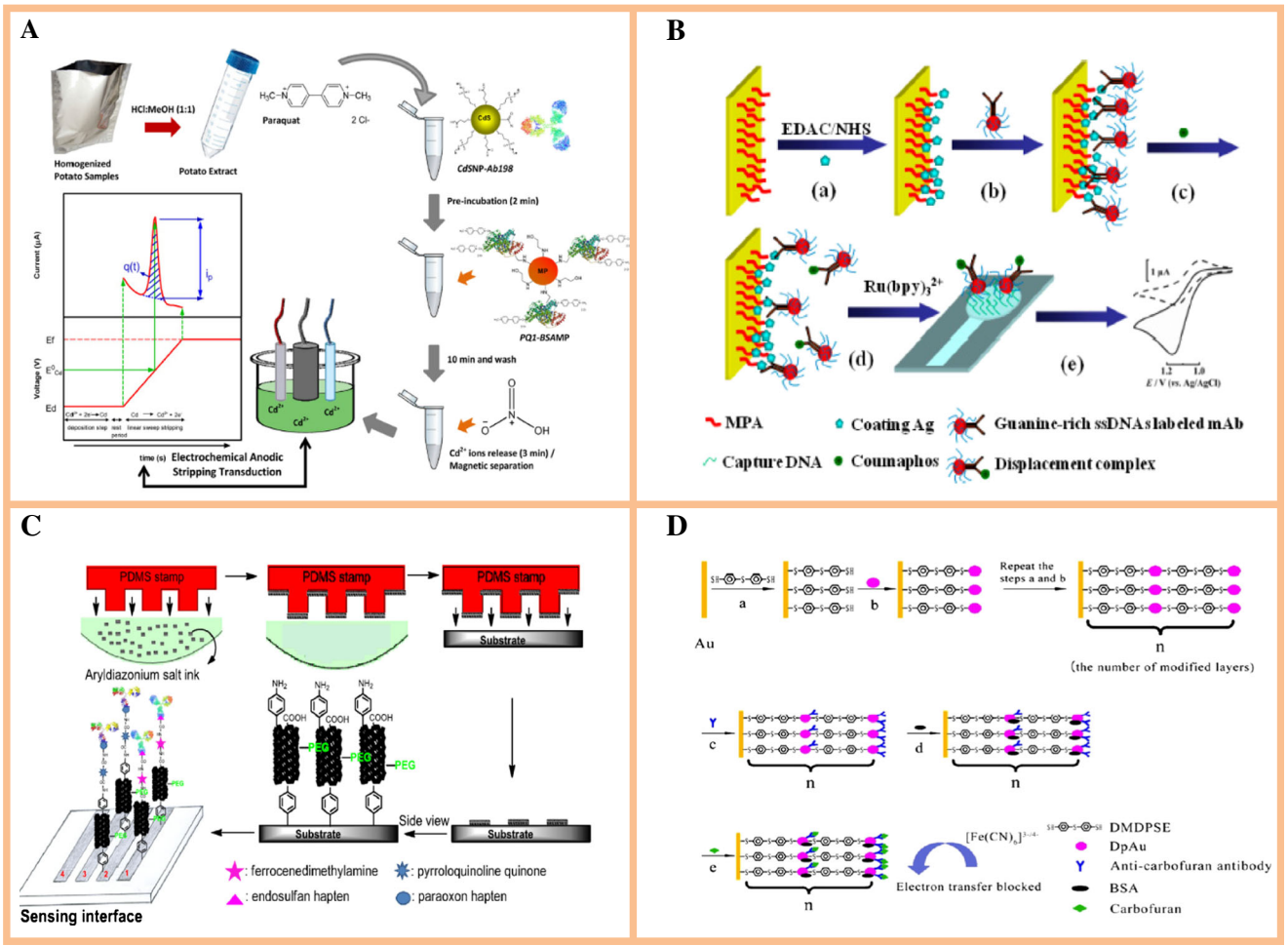

Fig. 3 Examples of immunosensors. Schematic diagram of **a** the immunosensor measurement procedure for paraquat detection using the CdS as immunocomplex label [Reprinted with permission from reference 52]; **b** the immunosensor for coumaphos detection using a label free displacement format [Reprinted with permission from reference 53]; **c** the immunosensor array for simultaneous detection of endosulfan and paraoxon using a label free displacement format [Reprinted with permission from reference 54]; **d** the immunosensor for carbofuran detection relying on the exploitation of the large surface area of multilayer approach. The immunocomplex formation was estimated measuring decrease of ferro/ferricyanide peaks [Reprinted with permission from reference 55]

immobilized antibodies. For instance, in the detection of small compounds as coumaphos, immunoassays are mostly restricted on a competitive format, exhibiting their intrinsic limitation for high throughput sensing systems. To overcome this limitation, Dai et al. developed a novel immunosensor based on an electrochemical displacement immunoassay coupled with oligonucleotide for coumaphos detection at attomolar level [53]. In details, antigen-modified gold substrate was used to immobilize guanine-rich single strand DNA-labeled antibody specific for coumaphos. Since the binding affinity of coating antigen to antibody is 1346-fold lower than that of coumaphos, the anchored guanine-rich single strand DNA-labeled antibody is displaced rapidly and efficiently after the addition of the analyte by the formation of a displacement complex (Fig. 3b). Coumaphos was sensitively determined by the enhanced catalytic cycle of guanine-Ru(bpy_3)$^{2+}$ with a limit of detection down to 0.18 ng L^{-1} (4.96×10^{-13} M). This immunosensor was also challenged in milk samples, and the achieved recovery values were in the range from 93.6 % to 98.2 %, with RSD values between 4.9 % and 6.6 %, indicating satisfactory accuracy levels.

To develop a label-free immunosensor, a novel multianalyte electrochemical immunosensor based on the assembly of patterned SWCNTs on glassy carbon was developed for the simultaneous detection of endosulfan and paraoxon. The structure of SWCNT was exploited to create a forest of SWCNTs patterned on glassy carbon, which provided an interface for efficient electron transfer between biomolecules and electrodes. Then redox molecules ferrocenedimethylamine and pyrroloquinoline quinone were attached to the SWCNTs, respectively followed by the attachment of specific antigens and antibodies (Fig. 3c) [54]. The electrochemical response of the redox probes is modulated by the binding between the antibody and the antigen. Based on

different electrochemistry signals from the two redox probes, this fabricated immunosensor array was used to simultaneously detect two analytes (endosulfan and paraoxon) in one sample. The immunosensor was characterized by a detection limit of 0.05 μg L^{-1} (1×10^{-10} M) for endosulfan and a detection limit of 2 μg L^{-1} (7×10^{-9} M) for paraoxon. The immunosensor was also tested in drinking waters with a recovery of 95 % ca.

A different strategy can be employed to develop label free immunosensors, relying on the exploitation of the large surface area of multilayer approach. For this purpose, gold nanocrystals and thio-bis-benzenethiol were used. In details, gold nanocrystals were assembled on thio-bis-benzenethiol modified gold electrode to immobilize carbofuran antibodies (Fig. 3d) [55]. In order to improve the binding capacity of antibodies and enhance the detection sensitivity, gold nanocrystals and thio-bis-benzenethiol were self-assembled by layer-by-layer technology to form multiple membranes through Au-S bond. The formation of the immunocomplex was estimated measuring the decrease of ferro/ferricyanide peaks because the electron transfer of ferro/ferricyanide is rather blocked in presence of the immunocomplex. A linear relationship in the detection of carbofuran up to 1.0×10^6 ng mL^{-1} (with a detection limit of 0.06 ng mL^{-1} equal to 3×10^{-10} M) was achieved, and several real samples like lettuces, cabbages, green peppers, tomatoes, Chinese chives and strawberries were analyzed, obtaining recovery values from 82 to 109.2 %.

Considering the above mentioned works, it is evident that the use of nanomaterials in immunoassay development has intrinsic advantages, being able to overcome the drawbacks of conventional immunosensors, including multiplex washing steps, extended enzymatic reaction time, and single analyte detection. These results attained a rapid evaluation of the immunocomplex and thus a smart detection of the target analyte.

Aptamer-based biosensor

Aptamers are short nucleic acids or peptide-based artificial molecules, often termed as chemical antibodies, with specific binding affinity towards their target analytes. They can be isolated from chemically synthesized combinatorial libraries by Selection Evolution of Ligands by Exponential enrichment (SELEX), produced by chemical synthesis, and purified to a very high degree [56].

Aptamers possess several competitive advantages over antibodies, including accurate and reproducible chemical production. They are also stable in extreme conditions, and easy to be modified with functional groups. Moreover, immunization and animals hosts are not required for their production. Thanks to these advantages, numerous aptamer-based biosensors have been devised for the detection of a wide range of target analytes [57]. In the review of Sassolas and colleagues [58] entitled "Biosensors for Pesticide Detection: New Trends" published in 2012, the authors reported the following: "*Recently, a DNA aptamer specific for acetamiprid was described. The potential of aptamers for the pesticide detection has not still been exploited but aptamer-based biosensors could be an alternative to the conventional methods of pesticide analysis*". This sentence highlights the importance of the discovery of this aptamer [59] that demonstrated a high potential for pesticide detection. Indeed, this aptamer has been further widely employed by many research groups for the construction of biosensors to detect acetamiprid at low level. The first example was reported by Fan et al. [60], which immobilized the aptamer onto a gold electrode modified with gold nanoparticles. The resulting formation of acetamiprid-aptamer complex was evaluated by using the electrochemical impedance spectroscopy (EIS), a label free technique widely used in biosensor field in the last few years [61]. EIS evaluates the impedance changes of the electrode surface and thus the resistance to electron transfer of the redox probe between the solution and the electrode, obtaining information about changes of the electrode surface. The charge transfer resistance (R_{ct}) is the usually calculated parameter, which is estimated according to the diameter of the semicircle present at the high frequency region in the Nyquist plot. With this configuration, the authors evaluated the formation of acetamiprid–aptamer complex following the increasing of R_{ct} value, as shown in Fig. 4a. A wide linear range was attained from 5 to 600×10^{-9} M with a low detection limit of 1×10^{-9} M. The applicability of this aptasensor was successfully evaluated by determining acetamiprid in tomatoes [60].

Recently, with the aim to enhance the biosensor sensitivity, Fei and collaborators assembled an aptasensor within a gold nanoparticles decorated multi-walled carbon nanotube-reduced graphene oxide nanoribbon composite as support for aptamer immobilization [62]. By employing the resulting composite, an ultrasensitive label-free electrochemical impedimetric aptasensor for acetamiprid detection was obtained (Fig. 4b). This aptasensor displayed a linear response for acetamiprid in the range from 5×10^{-14} M to 1×10^{-5} M with an extremely low detection limit of 1.7×10^{-14} M. To demonstrate the practicality of this aptasensor, recovery tests were carried out by adding different concentrations of acetamiprid into water samples, and the recoveries ranged from 96.0 % to 106.6 %, indicating that it can be applied for analysis of real samples.

These previous examples demonstrated the possibility to replace antibodies with aptamers, with advantages in terms of easy manufacturing procedure, without animal use, freedom to introduce chemical modifications for further improvement, as well as robustness, specificity, and affinity as demonstrated by the low detection limits achieved. On the other hand, since specificity and sensitivity of aptasensors depend

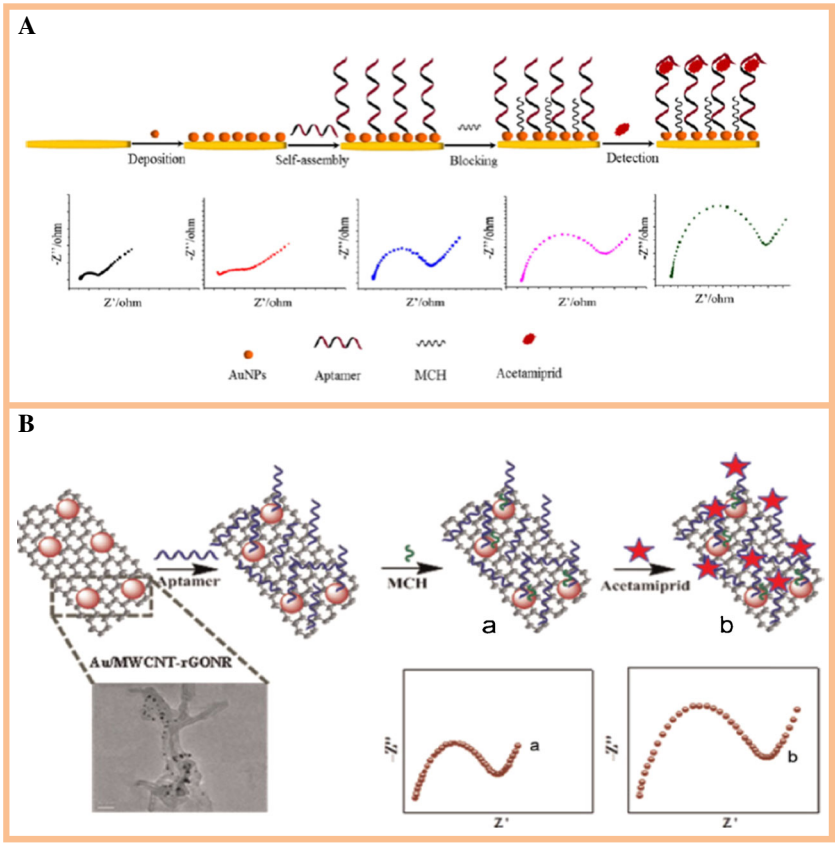

Fig. 4 Examples of aptasensors. **a-b** Schematic representation of the impedimetric aptasensors fabrication and principle for acetamiprid determination [Reprinted with permission from references 60, 62]

from the aptamer sequence ad hoc designed for the specific target, further research should be devoted to the design and synthesis of novel aptamers for the detection of a wider range of contaminants.

Nanomaterials

Nowadays, emerging nanomaterials have been employed to develop biosensing tools with custom-made properties and controlled nanoscale, including spheres and particles (metal nanoparticles, magnetic beads, quantum dots), nanotubes, nanowires, nanorods, nanofibers, as well as nanocomposites, nanofilms, nanopolymers, and nanoplates. These nanostructured materials have unique and special physical, chemical, mechanical, magnetic, and optical properties, such as strong absorption band in the visible region, high electrical conductivity and good mechanical features. This lead to the development of biosensors with enhanced detection performance in terms of response time, higher storage/operational stability, resistance toward environmental conditions, improved selectivity, reduced sample volumes, and easy sampling, as documented by the huge literature reported in the following paragraphs. Due to the rising of carbon-based nanomaterials, and their large use in this field, we divided this section in two main areas of interest: biosensors obtained by using carbonaceous-based modifiers (both pristine and composites) and biosensors modified with non-carbonaceous nanomaterials (i.e. noble metal nanoparticles, metal oxide nanoparticles, quantum dots).

Carbonaceous nanomaterials

Carbon nanotubes-based biosensors

Since their discovery in 1991 [63] carbon nanotubes have encountered tremendous interest in chemistry, physics and material science due to their 1-D structure and unique properties. These tubular structures, composed by sp^2 carbon units, can exist principally as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Thanks to their fascinating electronic, thermal, and adsorption properties, electroanalysis takes advantage of carbon nanotubes to improve the performance of the analytical methods towards pesticide detection. Oliveira et al. [64] described the use of MWCNTs paste (60:40 %, *w*/w, MWCNTs and paraffin binder) to immobilize laccase enzyme and fabricate a high-performance electrode for pirimicarb detection in vegetables. By replacing graphite with MWCNTs in a carbon paste electrode, a significant improvement of the current (6-fold) and an electrocatalytic effect has been obtained, for the detection of the substrate (4-aminophenol). Thanks to the presence of such

nanomodifier, pirimicarb was determined with a limit of detection of 1.8×10^{-7} M with a linearity range of 9.9×10^{-7}-1.15×10^{-5} M. Acceptable recoveries (from 90.2 ± 0.1 % to 101.1 ± 0.3 % for tomato, and from 91.0 ± 0.1 % to 100.8 ± 0.1 % for potato samples) were attained.

Although CNTs displayed a satisfactory performance, in literature their 1-D structure is often exploited in combination with other materials, e.g. metal nanoparticles, polymers, ionic liquids, quantum dots. For example, many kinds of polymers have been used in the development of sensitive CNT-based platform, such as polyaniline [35]. Furthermore, polyaniline was also enriched with polypyrrole to modify MWCNTs [65]. Here, the authors designed an AChE biosensor using the synthesized copolymer which provided a homogeneous and porous morphology ideal for enzyme entrapment. Electrochemical impedance spectroscopy revealed a decrease in R_{ct} because of the modification of the bare glassy carbon electrode with MWCNTs/polyaniline/polypyrrole. This result indicates that the electron transfer on the glassy carbon electrode modified with MWCNTs/polyaniline/polypyrrole was very fast. The use of MWCNTs made more effective the detection of the products from enzymatic reactions, achieving a limit of detection equal to 1.0 ng mL^{-1} (3×10^{-9} M) for malathion.

Poly(allylamine hydro-chloride) was also exploited for MWCNTs functionalization to immobilize AChE for monocrotophos detection. The modification of a carbon paste electrode with poly(allylamine hydro-chloride) functionalized MWCNTs allowed to get a current peak, due to enzymatic by-product (thiocholine) oxidation, at about 0.7 V (vs. Ag/AgCl). Due to the presence of poly(allylamine hydro-chloride)/MWCNTs, monocrotophos was successfully detected down to 0.88 pg mL^{-1} in cabbage, broccoli, and apple samples naturally contaminated [66].

Chitosan is one of the most employed biopolymers to develop nanomaterial based biosensors, since it can entangle with enzymes and nanomaterials, providing a favorable microenvironment to enzymes due to its good biocompatibility and excellent film-forming ability. For these reasons, chitosan has been coupled with a variety of materials together with CNTs. In their work, Zhai et al. [67] incorporated MWCNTs and hollow gold nanospheres into a hybrid film composed by chitosan and Prussian Blue to immobilize AChE for malathion, chlorpyrifos, monocrotophos, and carbofuran quantification. Voltammetric sensitivity and the R_{ct} of this biosensor were, respectively, higher and lower than those of the electrodes without MWCNTs or hollow gold nanospheres. The linear range and detection limit for malathion, chlorpyrifos, monocrotophos and carbofuran were found to be 0.05–75×10^{-9} M, 0.05–75×10^{-9} M, 0.1–50×10^{-9} M, 5–80×10^{-9} M and 0.05×10^{-9} M, 0.05×10^{-9} M, 0.1×10^{-9} M, 2.5×10^{-9} M, respectively. The recovery tests were studied by adding different amounts of pesticides into vegetables samples (cabbage, lettuce, leek, and pakchoi), respectively. The recoveries ranged from 94.2 % to 105.9 %.

Sun et al. [68] developed an amperometric immunosensor based on a nanocomposite formed by MWCNTs, thionine and chitosan, to detect chlorpyrifos residues. The formation of antigen and antibody complexes was performed using $[Fe(CN)_6]^{3-/4-}$ as redox probe and monitoring the decrease in the peak current before and after interaction between anti-chlorpyrifos antibody and chlorpyrifos. Chlorpyrifos was detected down to 0.046 ng mL^{-1} (1.3×10^{-10} M). In order to evaluate the feasibility of the proposed immunosensor for real sample analysis, cabbage, lettuce and Chinese chive samples were spiked with chlorpyrifos solutions of different concentrations, with recoveries in the range of 85.2 %-104.3 %. A similar detection limit for chlorpyrifos (0.05 ng mL^{-1} equal to 1×10^{-10} M) has been reported in the work of Chen [69] by using AChE immobilized on a nanocomposite formed by MWCNTs and SnO_2 nanoparticles kept in contact by chitosan.

Furthermore, MWCNTs have been also used together with α-cyclodextrin on a glassy carbon electrode. MWCNT/α-cyclodextrin composite was used to develop a potentiometric BChE-based biosensor as reported by Khaled et al. [70]. A SPE was modified with a MWCNT/polyvinylchloride composite incorporating α-cyclodextrin that worked as ionophore for the BChE substrate determination (butyrylcholine). The introduction of MWCNTs improved the electrode potential stability, leading to an enhancement of the sensitivity with respect to the bare electrode, and reaching a detection limit of 22 ng mL^{-1} (5.7×10^{-8} M) for ethion detection in commercial water samples.

While CNTs play a key role in detection, polymers have been more used for the entrapment of enzymes. Kesik et al. [71] synthesized a conducting polymer via electropolymerization, poly(4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)benzenamine), to immobilize the AChE enzyme. The combined unique properties of individual structures of MWCNTs and the polymer allowed a thiocholine detection at a low potential (0.1 V vs. Ag reference) due to a synergic effect. The electroactive surface area related to MWCNT/poly(4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)benzenamine) was 1.4-fold higher than that of MWCNTs and 3.4-fold higher than the one for bare graphite electrode. This platform resulted in 2.46 ng L^{-1} (8.94×10^{-12} M), 0.542 ng L^{-1} (1.86×10^{-12} M) and 4.90 ng L^{-1} (14×10^{-12} M) as detection limits, respectively for paraoxon, parathion, and chlorfenvinphos. This biosensor was challenged for pesticide detection in tap water and results were in agreement with HPLC analyses.

A variety of other materials has been also coupled to CNTs, due to their outstanding properties, such as conductivity and adsorption. Gan et al. [72] modified SPEs with AChE enzyme and a nanocomposite formed by CNTs, zirconia nanoparticles

and Prussian Blue, wrapped with Nafion. Dimethoate was detected down to 0.56 pg mL^{-1} using the Fe_3O_4/Au magnetic nanoparticles as enzyme carrier. Chinese cabbage samples were analysed using a standard addition procedure and compared to gas-chromatographic method with a recovery in the range of 88.0 % to 105 %. Considering these results, authors evidenced the important role of ZrO_2 nanoparticles related to their specific adsorption towards organophosphorus pesticides, also, the high conductivity intrinsically due to CNTs.

Ivanov et al. [73] reported on a single step modification of AChE-SPE using SWCNTs and Co phtalocyanine nanocomposite with the aim to decrease the working potential. SWCNT/Co phtalocyanine electrocatalyst displayed a synergic effect, increasing the efficiency of the platform. An experiment carried out with the same amount of Co phtalocyanine casted directly on SPEs, showed the need of higher potential for thiocholine oxidation (about 0.25 V vs. Ag/AgCl) in comparison with those obtained for SWCNT/Co phtalocyanine. This behaviour was ascribed to a better distribution characteristic of CNTs. Paraoxon and malaoxon were detected with detection limits of 3 ng mL^{-1} (1×10^{-8} M) and 2 ng mL^{-1} (6×10^{-9} M), respectively. Tap and sparkling waters were spiked with malaoxon and paraoxon and diluted with phosphate buffer before the measurement, and the results indicated some overestimation of the insecticide content. The authors explained this result as a sort of enzymatic activity loss due to the very low content of salts.

SWCNTs have been also employed to provide the assembly of patterned oriented antibodies on the glassy carbon substrate. In this configuration, SWCNTs are able to furnish a well-arranged orientation of the bioelements as well as an efficient electron transfer between biomolecules and the electrode. As an example, Liu et al. [74] assembled a forest of SWCNTs on glassy carbon substrate using a microcontact-printing technique. In detail, SWCNTs were covalently and vertically anchored on the electrode surface via the formation of amide bonds from the reaction between the amines located on the modified substrate and the carboxylic groups at the ends of the nanotubes. Ferrocenedimethylamine, as a redox probe, was subsequently attached to the ends of SWCNTs through amide bonding followed by the attachment of an epitope, i.e., endosulfan hapten to which an antibody binds. This architecture was able to provide a well assembled orientation of the haptens and consequently of the antibody. The use of such SWCNT-modified sensing platform allowed detecting endosulfan at 0.01 μg L^{-1} (2×10^{-11} M). A tap water sample was spiked with endosulfan and tested with recovery values of 93 %.

Graphene-based biosensors

Graphene and graphene-based nanocomposites, thanks to their remarkable 2-D configurations, allowed developing a plethora of biosensors towards the detection of pesticides in food samples. Zheng et al. [75] modified a glassy carbon electrode by using graphene functionalized with ionic liquid and gelatine. This nanocomposite, characterized by excellent conductivity and biocompatibility, promoted the electron transfer rate of the electrode interface and facilitated the adhesion of AChE due to the extremely hydrophilic surface. Because of the presence of ionic liquid/graphene with their inherent conductive properties the overpotential (oxidation of enzymatic product) was negatively shifted by 0.025 V vs. SCE, and the peak current was increased by about 140 % with respect to the biosensor without graphene, allowing carbaryl and monocrotophos to be detected down to 5.3×10^{-15} M and 4.6×10^{-14} M, respectively. Tomato juice samples were spiked with varying concentration of carbaryl and monocrotophos measured to evaluate the reliability of the proposed biosensor. Recoveries were found to be between 92.5 % and 105.0 % for carbaryl detection, and between 91.2 % and 110.0 % for monocrotophos detection, indicating acceptably accuracy for the analysis of food samples.

Oliveira et al. [76] developed a sensitive bi-enzymatic biosensor based on polyphenoloxidases-gold nanoparticles-chitosan hybrid film-graphene doped carbon paste electrode for carbamate detection. In detail, carbon paste electrodes doped with 20 % (*w*/w) of graphene was used as substrate to electrodeposit hybrid film composed of laccase, tyrosinase, and gold nanoparticles entrapped in a chitosan polymeric matrix, obtaining an enhanced conductivity of the platform. Using 4-aminophenol as substrate, the device presented wide linear ranges and low detection limits (1.68×10^{-9} - 2.15×10^{-7} M) to determine carbaryl, formetanate hydrochloride, propoxur, and ziram in citrus fruits based on their inhibitory capacity on the polyphenoloxidases activity. Recoveries at two fortified levels ranged from 93.8 ± 0.3 % (lemon) to 97.8 ± 0.3 % (orange).

The 2-D configuration of graphene was also exploited to develop several nanocomposites. Zhang et al. [77] developed an AChE biosensor based on a reduced graphene oxide/silver nanocluster/chitosan as glassy carbon electrode modifier. Reduced graphene oxide/silver nanocluster offered, with their excellent conductivity and catalytic effect, an ideal platform to fabricate an organophosphorus pesticide biosensor. To improve the stability of the biosensor, chitosan was used as a protective membrane. The biosensor was capable to linearly detect the phoxim from 0.2 to 250×10^{-9} M with a detection limit of 81×10^{-12} M. Using the standard addition method, the accuracy of the biosensor was evaluated in tap water samples observing recoveries in the range of 89.7 to 96.6 %.

Yang and co-workers [78] reported on a nanohybrid composed by gold nanoparticles, polypyrrole and reduced graphene oxide coupled with AChE for the detection of paraoxon-ethyl. Polypyrrole helped to avoid reduced graphene oxide aggregation and, together with gold nanoparticles, led

to a significantly increase of the surface area of the modified electrode, from 0.15 cm^2 (glassy carbon electrode) to 1.66 cm^2 (glassy carbon electrode modified with hybrid nanocomposite). The use of this nanocomposite allowed thiocholine detection at around 0.7 V vs. SCE, with an overpotential decrease of around 200 mV compared to that of the bare electrode. Paraoxon-ethyl was detected from 1.0×10^{-9} M to 5×10^{-3} M with a limit of detection of 0.5×10^{-9} M.

Wang et al. [79] modified a glassy carbon electrode through a drop-casting technique using a homogenous dispersion of zinc oxide nanoparticles and carboxylic graphene in Nafion. Successively, the modified sensor was further drop-cast with AChE and Chitosan solution to assemble the biosensor. The oxidation peak potential of thiocholine shifted to lower potentials (0.47 V vs. SCE). Detection limits for chlorpyrifos and carbofuran were obtained equal to 5×10^{-14} M and 5.2×10^{-13} M, respectively. To further demonstrate the reasonableness of the proposed method, recovery tests were performed on tap water by the standard addition method, observing recovery values in the range of 93.2–104.8 %. The low relative standard deviation (RSD = 5.9 %) for chlorpyrifos demonstrated the high precision of analysis.

Carboxylic graphene was also chemically modified with nickel oxide nanoparticles [80]. The authors dispersed the nanocomposite in Nafion, then drop-cast onto a glassy carbon electrode. This modified sensor was again drop-cast with AChE and chitosan solution to construct the biosensor. Also in this case the oxidation peak potential of thiocholine shifted to lower potentials (0.47 V vs. SCE), allowing the detection of carbofuran and chlorpyrifos at 10^{-13} M level, and methyl parathion at 10^{-14} M level. The same research group reported a similar work, employing SnO_2 instead of NiO [81], reaching also in this case detection limits at 10^{-13}/10^{-14} M level.

Wang et al. [82] developed a sensitive AChE amperometric biosensor based on the decoration of graphene with quantum dots made of CdS. The presence of CdS/graphene nanocomposite onto glassy carbon electrodes provided a sensitivity towards organophosphates about 3-fold higher than the unmodified glassy carbon electrodes. However, the applied potential was quite high, being equal to 680 mV vs. SCE. This biosensor was capable to detect carbaryl down to 0.7 ng mL^{-1} (3×10^{-10} M).

A nanocomposite formed by graphene and quantum dots was also subsequently adopted by Li and colleagues [83]. The authors deposited a nanocomposite based on CdSe@ZnS nanoparticles and graphene onto an indium thin oxide glass electrode in order to develop a novel photoelectrochemical AChE biosensor for paraoxon and dichlorvos detection at 10^{-14} M and 10^{-12} M. To demonstrate the feasibility of this biosensor, recovery studies in apple were performed, reaching a recovery from 93.6 ± 12.1 % to 103.3 ± 12.8 %.

Carbon black and others carbon-based biosensors

In spite of the predominant use of CNT- and graphene-based nanocomposites, the world of carbonaceous nanomaterials suggests a variety of alternatives, first of all carbon black (CB), a humble carbon nanomaterial that is being known for its outstanding properties [84–87] favorably compared with the most famous CNT and graphene. Several research groups demonstrated the high potential of CB to develop smart electrochemical biosensors for pesticide detection. This nanomaterial is characterized by carbon nanoparticle aggregates with diameters comprised between 17.95 and 32.5 nm [84]. Arduini et al. [88] developed a BChE inhibition biosensor based on SPEs for paraoxon detection. The group drop-casted SPEs with few microliters of a CB dispersion. Compared with the bare SPE, the use of CB allowed an increase of the sensitivity and a decrease in the overpotential, from 0.7 to 0.3 V (vs. Ag/AgCl). Indeed, the enzymatic hydrolysis of butyrylthiocholine over time was determined measuring the enzymatic product thiocholine at a working voltage of +300 mV, demonstrating the electrocatalytic properties of CB. The improved electrochemical performances can be ascribed to the high number of defect sites as well as to the better uniformly coverage of the working electrode when compared with working electrode surface modified with other nanomaterials like CNTs or graphene [84, 86]. The enzyme inhibition was linearly related to the concentration of paraoxon up to 30 μg L^{-1}, and the detection limit was 5 μg L^{-1} (2×10^{-8} M). The biosensor was stable for up to 78 days of storage at room temperature under dry conditions, being able to determine paraoxon in drinking water samples with a recovery value of 96 ± 2 %, demonstrating its accuracy in drinking water matrices.

Considering the results of Arduini et al. [88], it is important to highlight that the use of only CB as electrode modifier allows to thiocholine detection at lower applied potential than the use of nanocomposite consisting of several nanomaterials, such as the nanocomposite consisting of Prussian Blue nanocube/reduced graphene oxide nanocomposite reported by Zhang et al. [36], or the nanocomposite assembled with iron oxide and chitosan described by Jeyapragasam et al. [89].

Evtugyn's group also used CB as electrode modifier. In 2014 [90], they modified a glassy carbon electrode with a film of CB and silver nanoparticles decorated with macrocyclic ligand known as thiacalix [4]arene. Due to the electronic communication between CB and silver nanoparticles, the oxidation of thiocholine occurred at a very low potential of around 0.05 V (vs. Ag/AgCl). Furthermore, the presence of the macroclycle helped to prevent the direct oxidation of silver frequent in chloride-rich media. This platform allowed to accurately detecting malaoxon, paraoxon, carbofuran, and aldicarb, respectively down to 0.1, 0.05, 0.1, and 10×10^{-9} M. The AChE sensor was tested for the detection of residual

amounts of pesticides in spiked samples of peanut and grape juice. The limit of quantification in peanut extract was found to be 10×10^{-9} M for carbofuran and 20×10^{-9} M for aldicarb. This was much lower than tolerance level established for peanut by Code of Federal Regulations (1 ppm = 4.5×10^{-6} M of carbofuran, and 0.05 ppm = 0.26×10^{-6} M of aldicarb). The pesticides recovery in peanut showed values equal to 98–105 % for 10×10^{-9} M - 1.0×10^{-6} M carbofuran and 110–130 % for 20×10^{-9} M - 1.0×10^{-6} M aldicarb. The recovery values in grape juice for two concentrations of malaoxon (10 and 100×10^{-9} M) were equal to 95–98 %.

In a more recent work [91] Evtugyn's group developed a similar CB-based AChE biosensor by using the unsubstituted pillar [5]arene as an electron mediator onto glassy carbon electrodes. Using pillar [5]arene modified electrodes, the chronoamperometric detection of thiocholine enzymatic product was obtained at 200 mV. The efficiency of this platform provided detection limits equal to 4×10^{-12} M, 5×10^{-9} M, 0.02×10^{-9} M, and 0.6×10^{-9} M respectively for malaoxon, methyl-paraoxon, carbofuran, and aldicarb, resulting lower in respect of what obtained in the previous work [90]. In addition, the implemented sensor was challenged in spiked samples of peanut and beetroot. As highlighted by the authors, the advantages of pillar [5]arene relies in its well solubility in polar organic solvents (on the contrary of e.g. cobalt phtalocynine), giving a homogeneous mixture with CB and hence obtaining high reproducibility of the results.

Wei and Wang [92] used a nanocomposite obtained by combining honeycomb-like porous carbon, gold nanoparticles, and ionic liquid onto a boron doped diamond electrode to design an AChE biosensor for dichlorvos detection. The presence of the nanocomposite provided a better electric linkage and an acceleration of the electron transfer. By utilizing the porous carbon/gold nanoparticles/ionic liquid-boron doped diamond, sensitivity towards thiocholine resulted higher than 1.5- and 4.5-fold, if compared with porous carbon/gold nanoparticles/boron doped diamond and bare boron doped diamond. Dichlorvos was detected in a range from 4.5×10^{-13} to 4.5×10^{-9} M 10 with a detection limit of 2.99×10^{-13} M. The projected biosensor was employed to detect the content of dichlorvos in lettuce leaves sample, giving recoveries between 80.8 % and 93.1 %.

The same research group used other forms of carbon such as carbon spheres [16] and carbon aerogel [93]. In the last case the carbon aerogel was decorated by platinum nanoparticles to modify a boron doped diamond electrode to fabricate an AChE biosensor to detect methamidophos with a limit of detection equal to 3.1×10^{-13} M [93]. Also in this case, the biosensor showed satisfactory recovery values (between 91 and 106 %) in spiked apple juice samples.

Non-carbonaceous nanoparticles/rods/pores-based biosensors

Many platforms have been also developed avoiding the use of carbon-based nanomaterials. The use of noble metals [94] and metal oxide nanoparticles [14] (also nanowires, nanorods) have been largely involved in the realization of such diverse biosensors. These nanomaterials have been capable to improve biorecognition processes as in the immunoreactions, to be conjugated with biological elements, i.e. antibodies, nucleic acids, enzymes, to provide better electronic transduction and to stabilize/orientate the biosensing elements. Moreover, as previously reported [95, 96], the size of the nanoparticles represents a contributing factor on the orientation and activity of bioelements. For instance, Vertegel and coworkers [95] observed that the lysozyme's secondary structure was not significantly perturbed when the protein was adsorbed onto <20 nm-diameter silica nanoparticles. Many biosensors involving the use of different kind of nanoparticles have been adopted in pesticide monitoring research. Pichetsurnthorn et al. [97] designed an atrazine EIS-based biosensor comprising of a nanoporous alumina membrane integrated with a printed circuit electrode. The use of the nanoporous alumina established a high density array of confined areas. Binding of the pesticide to the anti-atrazine antibody has been measured by changes of the impedance associated with the perturbation to the capacitance of the electrical double layer. The device allowed to reach a detection limit in the fg mL^{-1} regime with a dynamic range spanning from 10 fg mL^{-1} (5×10^{-14} M) to 1 ng mL^{-1} (5×10 M).

Wei et al. reported [98] an amperometric immunosensor fabricated by modifying a SPE with a nanocomposite based on platinum colloid and silica sol-gel hybrid matrix. A competition immunoassay strategy was employed to determine chlorpyrifos-methyl by conjugating chlorpyrifos-methyl antigen to platinum colloid and using a horseradish peroxidase as antibody label. The use of Pt colloid led to an increase of the electrochemical response of 5 times, due to the electron transfer properties of Pt NPs. The dual signal amplification provided a limit of detection equal to 22.6 ng L^{-1} (6.45×10 M). Nesakumar et al. [99] developed an electrochemical inhibition biosensor based on AChE to detect captan in apple samples using, as electrode modifier, ZnO nanorod. The results were statistically used to find out the appropriate model for determination of captan, showing a linear range from 0.05 to 25.0×10^{-6} M with a detection limit of 107×10^{-9} M demonstrating also in this case that metallic nanoparticles improved the sensitivity. The same research group developed a pesticide biosensor by exploiting the favorable properties of ZnO in a brand-new configuration. Nesakumar et al. [100] modified a platinum electrode with ZnO nanocuboids, AChE and chitosan. These ZnO cuboids are characterized by a mean length of 398.24 ± 57.0 nm and a mean height of

163.28 ± 28.86 nm. As claimed by the authors, the presence of the ZnO-based matrix not only is powerful to assist the electronic communication between AChE and the electrode, but it also provides an effective microenvironment for boosting the enzyme-substrate interaction. After AChE was inhibited for 20 min, this biosensors allowed carbosulfan to be detect, in rice sample, down to 0.24×10^{-9} M with satisfactory recoveries (99.06–100.96 %). Arduini and colleagues [40] took advantage of ferric hexacyanoferrate, also known as Prussian blue (PB), as carbon-based SPE modifier since PB is commonly used to catalyze both the reduction of hydrogen peroxide and the oxidation of thiol-containing molecules. For this aim, PB nanoparticles have been utilized, together with BChE, to readily detect the given electroactive by-product (thiocholine) of the enzymatic reaction between butyrylthiocholine and BChE. The effectiveness of PB was also exploited by Wu et al. [101] in the development of an amperometric biosensor to detect monocrotophos based on AChE inhibition. A glassy carbon electrode was modified with a nanocomposite formed by AuNPs, poly(dimethyldiallylammonium chloride) (PDDA) and PB. Authors controlled the surface hydrophilicity by modulating the ratio of gold nanoparticles to PDDA-PB, indeed the static water contact angle of the modified surface was tailored from 25.6° to 78.1°. AChE was finely oriented onto this surface with significantly improved stability and activity. Thanks to that, AChE was more affine to the substrate (apparent Michaelis–Menten constant equal to 0.3 mM) if compared with other work where AChE was adsorbed on surface no hydrophobically tailored, i.e. AuNPs silica sol-gel composite reported by Du et al. [102]. The organophosphorus pesticide monocrotophos was detected with a detection limit of 0.8 pg mL^{-1} L (4×10^{-12} M) and this biosensor was challenged in garlic samples with recovery values from 92.8 % to 106 %. The combination between noble metals opportunely nanoscaled and hydrophobic polymer was recently reported by Turan and colleagues [103] in order to detect paraoxon in milk and tap water. A graphite electrode was firstly modified with poly(5,6–3 bis(octyloxy)-4,7-di(thieno[3,2-b]thiophen-2-yl)benzo[c] [1, 2, 5]oxoadiazole) (PTTBO), which provided an hydrophobic micro-environment to enhance BChE activity. Subsequently the charge transfer rate of the biosensor was improved through the deposition of silver nanowires on the polymer coated surface. After 5 min of incubation, paraoxon was able to inhibit the enzyme activity allowing to be detected down to 0.212×10^{-6} M, when butyrylthiocholine iodide was used as the substrate. Zapp et al. [47] switched to platinum in order to develop a sensitive biosensor for the determination of methomyl in carrot and tomato. They exploited the inhibition of laccase, which was immobilized in a new supported ionic liquid phase based on the combination between PtNPs and the ionic liquid known as 1-butyl-3 methylimidazolium tetrafluoroborate ionic liquid ($BMI{\cdot}BF_4$) supported on montmorillonite (clay mineral). This composite was subsequently used to fabricate a carbon paste electrode. The incorporation of the ionic liquid into the electrode led to a decrease in the resistance and an increase in the rate of charge transfer due to the high conductivity of this material. Furthermore, also PtNPs facilitate the electron transfer. Authors took advantage of this synergic catalytic contribution to detect linearly methomyl with a detection limit of 2.35×10^{-7} M and quantification limit of 7.8×10^{-7} M. The improved response using gold electrode modified with AuNPs to design a laccase based biosensor for formetanate hydrochloride detection was reported also by Ribeiro et al. [48]. By impedimetric experiments, the authors evaluated the improvement of the conductivity of the platform upon AuNPs integration, by effectively lowering the resistance to charge transfer until 350 Ω (starting from and 960 Ω when AuNPs were absent). To detect monocrotophos in garlic sample, Wu et al. [104] used a mesoporous material known as mesocellular silica foam (MSF). This modifier not only served to provide a confined nanometric pores for AChE immobilization, preventing enzyme unfolding and enhancing its activity, but also acted as concentrating surface for pesticide through both physical adsorption and hydrogen bond. MSF containing AChE was attached to a glassy carbon electrode (GCE) by using poly(vinyl alcohol) (PVA) as polymeric membrane. The use of MSF-AChE-PVA/GCE allowed increasing the monocrotophos adsorption up to 3 times with respect of AChE-PVA/GCE. The resulted biosensor, after 10 min of incubation with pesticide, showed extremely high sensitivity, lowering the detection to 0.05 ppb (2×10^{-11} M) and expanding the linearity up to 10 ppb (4×10^{-8} M). Nanomaterials such as metal nanoparticles have been also largely used in the development of immunoassay to detect different kind of pesticides in food matrices as in the case of the immunosensor for ultrasensitive coumaphos detection using a gold substrate modified with AgNPs [53]. In the same context, Liu et al. [105] developed a label-free immune platform for atrazine detection in maize samples. A gold electrode was coated with 28 nm-diameter AuNPs by means of self assembled monolayer strategy. Consequently, the anti-atrazine monoclonal antibody was immobilized onto the gold layer. Following the addition of atrazine, the differential pulse voltammetric peak rapidly decreased with increasing concentration of the pesticide. The authors exploited the interaction between atrazine and anti-atrazine antibody, which interrupted the electron flow from the redox center of anti-atrazine and the electrode surface. The hindering of the electron transfer allowed the immunosensor to detect atrazine with a limit of detection of 0.016 ng mL^{-1} (7.4×10^{-11} M). Jia and colleagues [106] exploited the sensing properties of AuNPs in developing an impedance immunosensor to detect chlorpyrifos residue in vegetable samples, i.e. cucumber, lettuce. The authors utilized a gold interdigitated array microelectrode (IDAM) integrated into a microchannel of microfluidic chip.

The platform was obtained by modifying the gold surface firstly with PDDA and a layer of AuNPs. Then, the anti-chlorpyrifos monoclonal antibody was finely immobilized onto the IDAM surface through the use of protein A. The impedance measurements were conducted in the presence ferri/ferrocyanide mixture, allowing to detect the pesticide with a linear relationship between the relative impedance change and logarithm of chlorpyrifos concentration in the range of 0.5–500 ng mL^{-1}, reaching a detection limit of 0.5 ng mL^{-1} (1×10^{-9} M).

Sample treatment and real sample analysis

Sample treatment in food analysis is a crucial issue to accurately detect pesticides. Generally, treatment procedures require several steps, including sampling, extraction of targets from the samples, clean-up, and analysis. Considering the high complexity of food matrices, it is crucial the evaluation of lipid constituents, sample size (liquid, granules, powders), and sample morphology (meats, tissues, fruits, vegetables), in order to choose the right treatment approach. Clean-up procedures are also required in case of interfering species for their removing without affecting the pesticide recovery.

The most used methodologies for pesticide extraction and concentration in food are: liquid-liquid extraction, solid phase extraction, solid phase micro extraction, supercritical fluid extraction, matrix solid phase dispersion, accelerated solvent extraction; stir bar sorptive extraction, membrane assisted solvent extraction, and single drop microextraction [107]. In case of pesticide detection by means of biosensors, a simple treatment for a comprehensive detection is mandatory to obtain in field and easy measurements.

Tap water sample analysis requires simple pre-treatment, usually consisting of the dilution of the samples with buffer solutions. For instance Ivanov and coworkers [73] provided a simple dilution of tap and sparkling water samples, spiked with malaoxon and paraoxon, with phosphate buffer solution and then measured their inhibitory effects on AChE by amperometry. While Arduini and colleagues [88] diluted drinking water samples 1:2 (*v*/v) with phosphate buffer 0.1 M + KCl 0.2 M for successive electrochemical analyses of paraoxon samples.

More complex liquid matrices, such as juices, necessitate of different treatment steps including pH adjustment to 7.3 by 0.1 M NaOH and filtration through a 0.22 μm filter, to guarantee a correct pesticide extraction procedure [93].

Alternatively, solid matrices, due to their high complexity, require a huge number of treatment steps for homogenizing the sample to achieve a liquid extract to be successively analyzed. For instance, apple and carrot samples require sample chipping, centrifugation at 37 °C for 20 min at 8000 rpm, supernatant filtration through a 0.2 μm PVDF filter, and dilution with distilled water, as reported by Bao et al. for organophosphate detection using a plant esterase biosensor [43].

Fan et al. [60] treated tomato samples by homogenization in a mortar, centrifugation for 20 min at 1000 rpm to remove the solids, and successive filtration through a 0.22 μm membrane, to analyze acetamiprid using aptasensor.

Organic solvents are also used for sample treatment but requiring evaporation to avoid the activity loss of the bioelements. Gan et al. [72] treated Chinese cabbage sample by homogenization in a mortar, extraction with 20 mL of acetone, centrifugation for 5 min at 3000 rpm, and evaporation by rotary evaporation to 1.0 mL.

A different approach for sample treatment has been developed by Anastassiades, Lehotay, Stajnbaher, and Schenck [108] combining the extraction/isolation of pesticides from food matrices and extract clean-up. This methodology was termed Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) and employ acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide multi-residues with good recovery rates [109]. Nowadays, this method is widely used for food analysis because it combines several steps, which can be tailored for specific requirements, and extends the range of pesticides recovered with respect to classical extraction techniques.

Samples of mango and grape have been treated by using this method as reported by Ribeiro et al. [48]. Fruit samples were chopped and homogenized in accordance with the guidelines of the European Council Directive [110] and subsequently extracted by QuEChERS. In detail: i) aliquots of homogenized samples were quantitatively transferred to a QuEChERS tube containing buffer-salt mixture; ii) acetonitrile was added and the tube shaken and centrifuged for 5 min at 4500 rpm; iii) solvent layer was reduced by evaporation under vacuum and transferred onto a column clean-up again shaken and centrifuged as mentioned above; iv) supernatant was then evaporated to dryness and, immediately before the analysis, the residue was re-dissolved with the supporting electrolyte.

As noticed by the detailed description of QuEChERS procedure, this method is rather more complex and tedious with respect to the treatment procedures above described. However, this method has the main advantage that positive samples can be further analyzed using standard chromatographic procedures, without any additional clean-up step.

As highlighted above, simple matrices like water require easy pre-treatment protocols while more complex matrices necessitate ad hoc pre-treatment to obtain low matrix effects and high percentage of recovery. In addition, tailored pre-

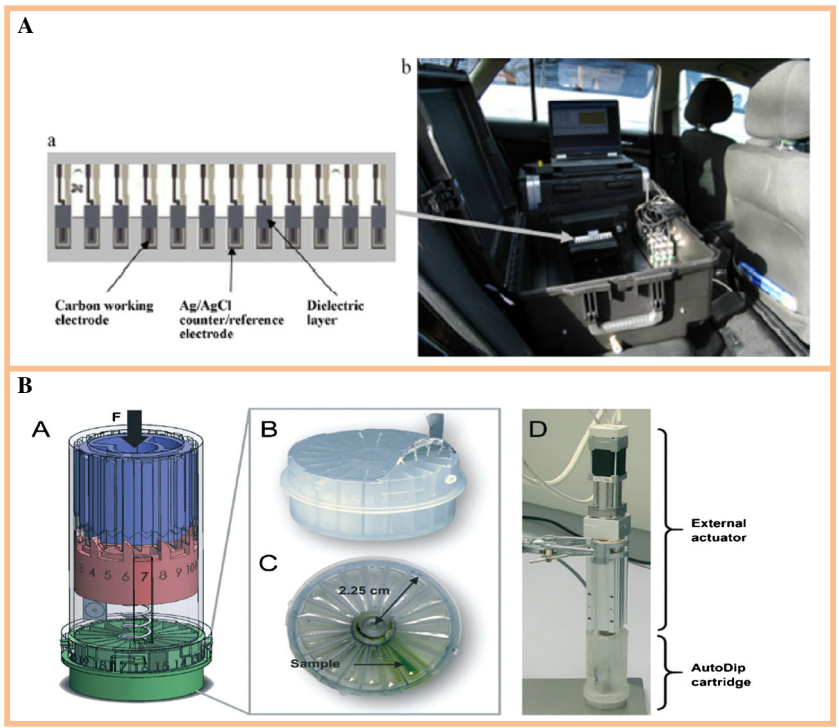

Fig. 5 Examples of vanguard technologies in tailor-made food analysis **a** AChE biosensor array comprising 12 screen-printed carbon electrodes for organophosphorus pesticides (a); array embedded in the prototype biosensor system operating in the field powered from a car battery via the lighter socket (b) [Reprinted with permission from reference 113].; **b** Detailed AutoDip design for minimal sample preparation and an integrated reagent pre-storage module coupled with AChE biosensor for chlorpyrifos-oxon detection. In detail, 3D drawing of the AutoDip cartridge (A), image of the reagent module fabricated by stereolithography and sealed with peelable foil (B), top view of the reagent module pre-filled with reagents and a homogenized food sample (green) (C), prototype of the AutoDip cartridge with an external vertical actuator (D) [Reprinted with permission from reference 114]

treatment protocols, for the extraction of food samples analyzed with biosensors, can be configured to be compatible with standard laboratory analytical methods for the analysis of samples resulting positive in field avoiding further treatments.

Although several biosensors have been applied in real samples, a specific validation of these biosensors in comparison with standard laboratory techniques (e.g. chromatography) is reported in limited literature. A proper in-house validation should include the evaluation of key analytical biosensor parameters, such as selectivity/specificity, trueness by recovery at three level of concentration, repeatability and within-laboratory reproducibility at three level of concentration, instrumental/method detection limits (LoDs) and quantification limits (LoQs), range of linearity, standard measurement uncertainty, stability studies in different storage conditions [111]. Very few articles are reported in literature describing a detailed validation of the biosensors in comparison with laboratory set-up methodologies. Among them, Cesarino et al. [35] described the evaluation of the analytical performances of the implemented biosensor not only in terms of linear range, detection limit, stability, reproducibility, and repeatability, but also in terms of comparison with HPLC data using Student's t-test, highlighting any significant differences at a 95 % confidence level.

Vanguard technologies in tailor-made food analysis

The exploitation of nanomaterials functionalized with biocomponents in the design of analytical devices can dramatically improve the stability and specificity, as well as reproducibility and reliability of the detection system. Moreover, nanostructured materials and bioelement integration, in combination with the miniaturization of the transducer, allow all the components to be embedded into an ad hoc device. These integrated systems may lead to perform high throughput analyses for rapid and low cost screening of pesticides in a wide variety of complex matrices, using small sample volumes.

This overall scenario of emerging technologies has encouraged researchers to envisage alternative strategies for the development of tailor-made electrochemical biosensors for food analysis.

As an example, several efforts have been spent towards the evolution of the printed technology to produce biosensor strips for miniaturized portable instruments, expanding the

implementation potential of SPEs in real life applications, including food analysis. Indeed, the peculiarity of disposable SPEs is observed by the huge possibility for mass production, allowing easy customization, effective portability, cost-effectiveness, low sample volume requirement, and considerable application directly on field [112].

In this context, a number of biosensors based on SPEs has been designed for applications in food analysis to monitor toxic compounds (e.g. pesticides, heavy metals, pathogens and toxins, explosives, phenols), including also alarm systems to reveal hazardous conditions in industry or on farms. A crucial example of integration and system embedding printed technology with sensing and ICT tools is that of Crew and coworkers [113], which designed an automatic system for the rapid organophosphorus pesticide identification and quantification in food samples, consisting of an electrochemical biosensor array developed using screen-printing technology (Fig. 5a). The authors conceived this system according to the end-user requirements in terms of reliability but also low cost and mass production. The miniaturization of the system due to the use of printed electrodes, allowed to easy construct an array of six AChE enzymes to measure pesticides by chronoamperometry at a potential of 0 V vs. Ag/AgCl. This embedded system was also validated for food analysis; a wide selection of raw foods were spiked with six different pesticides and analyzed. To report an example, recovery studies for chlorfenvinphos at concentrations of 10^{-5} M and 10^{-7} M gave values of 78 % and 93 %, respectively.

On the other hand, microfluidics and lab-on-chip technologies hold promise for automation of sampling and analysis with minimal consumption of reagents in portable devices, in comparison with flow-based biosensor systems. Indeed, a microfluidic platform approach represents a crucial requirement for the integration and automation of a biosensing event within a suitable advanced instrument capable of real sample handling. However, due to the use of small channels and flow cells, this approach suffers from the problem of complex food matrices, and is therefore limited to liquid samples (e.g. tap water, milk). In this context, Drechsel and colleagues [114] developed a novel "AutoDip" platform based on the movement of a solid phase through reagents and samples, instead of transporting a sequence of reagents through a fixed solid phase (Fig. 5b). The authors applied this platform for the electrochemical detection of organophosphorus pesticides in real food samples using an AChE biosensor, obtaining a minimal sample preparation and an easy handling of the assay, thanks to integrated reagent pre-storage modules. Detection of chlorpyrifos-oxon spiked into apple samples at concentrations of 10^{-7} M was reported, according to the maximum residue level for chlorpyrifos in apples defined by the European Commission.

Besides these novel technologies allowed for the development of smart sensing systems, further research is still

Table 1 Some examples of nanomodified biosensors for pesticide detection in food samples

Analyte	Electrode modifiers	Bio-component	Technique	LOD	Linear range	Sample	Ref
Aldicarb	CB, pillar [5]arene	AChE	CA	0.6×10^{-9} M	7×10^{-9} M - 10^{-5} M	Peanut	[91]
Atrazine	AuNPs	Anti-atrazine Ab	DPV	0.016 ppb (74×10^{-12} M)	0.05–0.5 ppb (0.23–2.3 × 10^{-9} M)	Maize	[105]
Captan	ZnO nanorod	AChE	CA	107×10^{-9} M	0.05–25.0 × 10^{-6} M	Apple	[99]
Carbaryl	G, IL	AChE	CA	5.3×10^{-15} M	10×10^{-15} M - 10^{-8} M	Tomato juice	[75]
Carbosulfan	ZnO nanocuboids, chitosan	AChE	CA	0.24×10^{-9} M	5–30 × 10^{-9} M	Rice	[100]
Chlorfenvinphos	MWCNTs, poly(SNS-NH_2)	AChE	A	4.9 ppt (14×10^{-12} M)	0.005–0.1 ppb (14–300 × 10^{-12} M), 0.1–12.5 ppb (300 × 10^{-12} M − 35 × 10^{-9} M)	Tap water	[71]
Chlorpyrifos	MWCNTs, thionine, chitosan	Anti-chlorpyrifos Ab	DPV	0.046 ppb (0.13×10^{-9} M)	0.1–10^{5} ppb (0.3 × 10^{-9} M-0.3 × 10^{-3} M)	Cabbage, lettuce, Chinese chive	[68]
Coumaphos	AgNPs	ssDNA-mAb	CA	0.18 ppt (0.5×10^{-12} M)	0.5–80 ppt (1.4×10^{-12} M-0.22 × 10^{-9} M)	Milk	[53]
Dichlorvos	CdSe@ZnS NPs	AChE	PE	10^{-12} M	10^{-12} M - 10^{-6} M	Apple	[83]
Dimethoate	CNTs, ZrO_2NPs, PB, Nafion	AChE	DPV	0.56 ppt (2.4×10^{-12} M)	1 ppt-10 ppb (4.4×10^{-12} M − 44×10^{-9} M)	Chinese gabbage	[72]
Endosulfan	SWCNTs	Endosulfan monoclonal Ab	SWV	0.01 ppb (25×10^{-12} M)	0.01–20 ppb (25×10^{-12} M-49 × 10^{-9} M)	Tap water	[74]
Ethion	MWCNTs, PVC, α-cyclodextrin	BChE	P	22 ppb (57×10^{-9} M)	0–330 ppb (0–858 × 10^{-9} M)	Commercial water	[70]
Formetanate hydrochloride	AuNPs	Lac	SWV	95×10^{-9} M	95×10^{-9} M-11.3 × 10^{-3} M	Mango, grapes	[48]
Malathion	MWCNTs, AuNPs, PB,chitosan	AChE	DPV	0.05×10^{-9} M	0.05–75 × 10^{-9} M	Cabbage, leek, lettuce, pakchoi	[67]
Methomyl	PtNPs, ($BMI{\cdot}BF_4$), montmorillonite	Lac	SWV	0.235×10^{-6} M	0.98–9.0 × 10^{-6} M	Carrot, tomato	[47]
Monocrotophos	AuNPs, PDDA, PB	AChE	A	0.8 ppt (3.6×10^{-12} M)	1–1000 ppt (4.5×10^{-12} M-4.5 × 10^{-9} M), 1–10 ppb (4.5–45 × 10^{-9} M)	Garlic	[101]
Paraoxon	SWCNTs, CoPc	AChE	CA	3 ppb (7×10^{-9} M)	5–50 ppb (18–180 × 10^{-9} M)	Tap water, sparkling water	[73]
Phoxim	rGO, AgNPs, chitosan	AChE	DPV	81×10^{-12} M	0.2–250 × 10^{-9} M	Tap water	[77]
Pirimicarb	MWCNTs	Lac	SWV	0.18×10^{-6} M	1–11.5 × 10^{-6} M	Tomato, potato	[64]
Ziram	G, AuNPs, chitosan	Lac, Tyr	SWV	1.68×10^{-9} M	100–338 × 10^{-9} M	Lemon, orange	[76]

required for their integration into lab-on-chip, probably constrained by the lack of open dialogs among specialists in different cross-disciplinary technologies.

Conclusions

According to the new World Report entitled "Sensors Markets 2016" (Intechno Consulting 2016), the non-military, open market for sensors grew from EUR 81.6 billion in 2006 to EUR 119.4 billion in 2011 and can be expected to grow to EUR 184.1 billion until 2016. Despite the considerable research on biosensor technology, their commercialization and consequently applications remain limited, since biosensors bring about a combination of challenges.

Latest trends in nanomaterial-based electrochemical pesticide assays for use in food analysis underpin the potential of nanoscale materials in the design of ad hoc sensor systems with improved sensitivity, stability, and reliability. Nanomaterials were used not only to improve the electrochemical response of the electroactive analytes, but also as labels to improve sensitivity and allow multi-analysis. Thanks to these key advantages, several electrochemical nanomaterial-based biosensors have been reported in literature for the detection of pesticides in food as broadly described throughout this review. A selection of these biosensors was reported in Table 1.

Nevertheless, although the high sensitivity and rapid response of electrochemical nanobiosensors, a number of drawbacks still make them challenging to be applied to real sample analysis, including robustness and storage stability, without requiring particular conditions of temperature and humidity.

In this context, advances in microfluidics, print technology, sample pre-treatment, and lab-on-a chip technology has resulted in effective pesticide sensors for use in the food supply chain from the farm to the fork**.** Further research on integration of sensors in networks, combined with wireless signal transmitters for remote sensing, capable to provide high-throughput detection will also enable sensor technology to move from laboratory to real applications in the field. Indeed, sensor technology has the potential to optimize the sustainability of agriculture and reduce its environmental impact by providing accurate analyses of pesticide concentration in soil and crops, as well as to improve human wellbeing ensuring safe and healthy food products.

CB: carbon black, AuNPs: gold nanoparticles, G:graphene, IL: ionic liquid, MWCNTs: multi-walled carbon nanotubes, poly(SNS-NH_2): poly(4-(2,5-di(thiophen-2-yl)-1*H*-pyrrol-1-yl) benzenamine), AgNPs: silver nanoparticles, PB: Prussian Blue, PtNPs: platinum nanoparticles, PDDA: poly(dimethyldiallylammonium chloride), SWCNTs: single-walled carbon nanotubes, CoPc: cobalt phthalocyanine, rGO: reduced graphene oxide, AChE: acetylcholinesterase; Lac: laccase, Ab: antibody; BChE: butyrylcholinesterase, Tyr: tyrosinase, A:amperometry, CA: chronoamperometry, DPV: differential pulse voltammetry, P: potentiometry, PE: photoelectrochemical, SWV: square wave voltammetry.

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Compliance with ethical standards Please check "Compliance with ethical standards" statement if presented correctly.ok The author(s) declare that they have no competing interests

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