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Review

Electrochemical biosensors in food safety: challenges and perspectives

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Abstract: Safety and quality are key issues for the food industry. Consequently, there is a growing demand to preserve the food chain and products against substances toxic, harmful to human health such as contaminants, allergens, toxins, or pathogens. For this reason, it is mandatory to develop highly sensitive, reliable, rapid, and cost-effective sensing systems/devices such as electrochemical sensors/biosensors. Generally, conventional techniques are limited by long time of analyses, expensive and complex procedures, and they require skilled personnel. Therefore, the development of performant electrochemical biosensors can significantly support the screening of food chain and products. Here, we report some of the recent developments in this area and analyze the contributions produced by electrochemical biosensors in the food screening and the challenges to address.

Keywords: food; safety; electrochemical biosensors; bacteria, toxins, pesticides, antibiotics, contaminants

1. Introduction

The food safety is an important critical issue for the modern food industry. Contaminants, bacteria, toxins, etc. can enter in the food chain during the production different steps. For example, they can accumulate in food during storage, and/or are produced in the food by reaction with chemical compounds [1].

A preventative approach to food safety is the HACCP (Hazard Analysis Critical Control Point), which attempts to avoid the contamination of unwanted substances into the food chain [2, 3]. On the other hand, some rigid guidelines are defined by the regulatory agencies such as United States Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA). All these guidelines indicate the maximum levels for contaminants in foods to preserve the consumer health [4,5].

Food analysis is carried out at the end of the production process using conventional techniques such as chromatography, mass spectrometry, ultraviolet detection, or fluorescence techniques either individually or in combination with other separation techniques [6,7]. These traditional approaches have several limitations. Firstly, since the analysis is performed at the end of the process, contaminated products can pass through the entire production chain or even they can be placed on the market before their contamination is noticed. Second, these methods of analysis are laborious and complex, expensive, time consuming, require large sample volumes, and skilled personnel [8]

In this context, biosensors can offer a possible alternative allowing a screening of food samples before the end of the production process [8]. Furthermore, biosensors also provide rapid and on-site monitoring, and a real-time information about the production process [9]. Among the various type of biosensors, electrochemical biosensors have been widely used due to their well-understood biointeraction mechanisms and detection process [10]. Being an accurate, sensitive, specific, and rapid analysis system, the

electrochemical biosensors can represent smart detection tools for food commodities [11,12].

In this review, we consider recently developed electrochemical biosensors applied for food analysis and safety. We will illustrate the recent advances in biosensing technologies and evaluate the related weakness and drawbacks. We will include some future ideas and challenges that the electrochemical biosensors must overcome to be as the new and smart tool for food analysis and safety.

2. Electrochemical biosensors

A biosensor is an analytical device used to determine the molecules amount in a sample. Generally, it is characterised by a bioreceptor (enzyme, whole cell, antibody, aptamer, nucleic acid) connected to a suitable transducer; the specific interaction between the target molecule and the biocomponent generates a physico-chemical or biological signal, converted into a measurable property by the transducer. The choice of the bioreceptor and the transducer depend on the characteristics of the sample and the type of measurable property to be considered. The bioreceptor represents the biosensor key element, responding only to a particular analyte and not to the interferences eventually present in the sample under analysis [13]. Biosensors can be classified in agreement with the type of recognition element or with the type of signal transduction [14,15]. As regards the first classification, biosensors are divided into two main groups: catalytic and affinity biosensors. In the first case, the recognition element can be characterized by enzymes, whole cells (bacteria, fungi, cells, yeast), cells organelle and plant or animal tissue slices; the catalytic sensors have the most consolidated tradition in the field of biosensors: historically, glucose biosensors are the most cited examples including a wide commercial success and diffusion [16]. Considering the affinity-based biosensors, the biomolecule can be represented by chemoreceptors, antibodies, nucleic acids, and they provide selective interactions with the analyte. [2, 17].

Concerning the transducers classification, a wide typology of transduction techniques has been developed in biosensing; in particular, the most common are optical, piezoelectric, calorimetric, and electrochemical and in this review the focus is on electrochemical ones. Very interesting and recent reviews have illustrated the characteristics and performances of the other biosensors with different transducer systems [1,3,18,19]

As concerns the electrochemical biosensors, measurements of signals from biological samples are generally linked to an electrochemical reaction, involving a bio element electrochemically active. Usually, biological reactions can generate either a change in conductance or impedance, a measurable current, or charge accumulation, measured by conductometric, potentiometric, or amperometric techniques [20]. Investigated reactions are normally detected near the electrode surface and the detection techniques are generally chosen considering the electrochemical properties of the electrode surface. Electrochemical techniques involve a reference, auxiliary, and a working/sensing electrodes.

The working/sensing electrode acts as a transduction element, whereas a counter electrode establishes a connection between the solution and the sensing electrode surface [21].

2.1. Electrochemical techniques

Electrochemical techniques have been considered useful tools for food safety analysis. They are cheap, portable, easy to handle, and fast, thus they can be preferred to the other analytical techniques. The electrochemical biosensors illustrated in this review are impedimetric, amperometric/voltammetric, and potentiometric ones; they are

classified by the measured signals, which are impedance/conductance, current, and p otential.

Here below a brief overview of the principal electrochemical techniques mentioned in this review. For more details about theories underlying the different electrochemical techniques, several books and reviews in the literature are well known [20, 22-27]

Cyclic Voltammetry (CV) and Linear Sweep Voltammetry (LSV)are among the most widely diffused electrochemical techniques and can be used to study the electrochemical behavior of molecules electrochemically active, measuring the current produced by the redox reaction varying the potential.

In a CV experiment, the potential of the working electrode is scanned linearly versus time, and after the scheduled potential is reached, the potential is scanned in the opposite direction to return to the initial potential. In the LSV experiment, the potential is scanned linearly versus time, but no return scan is performed.

Differential pulse voltammetry (DPV) and Square wave voltammetry SWV can be classified as pulse voltammetric techniques.

DPV and SWV in comparison with CV and/or LSV can be used to study the redox properties of electroactive compounds in very low amount for two main reasons: (1) the effect of the charging current can be minimized, achieving higher sensitivity and (2) only faradaic current is extracted, so electrode reactions can be analyzed more carefully.

Potentiometry (PM) measures the potential of a solution between two electrodes is used in electroanalytical chemistry to measure the electrochemical potential of charged species. However, a highly stable and accurate reference electrode is required, which could limit the application of PM in bioanalysis.

Constant potential amperometry (CPA) is an electrochemical technique in which the current is measured while the potential difference between the sensing and reference electrodes is held at a constant value sufficient to oxidize or reduce the analyte. This potential value is generally evaluated from the CV or LSV experiment

Chronoamperometry (CA) is a potentiostatic technique, where the current is recorded as a time function and it is useful to determine the concentration of the analyte, once its identity is known using other techniques such as CV, chromatography and/or other separation techniques.

Electrochemical impedance spectroscopy (EIS) is an effective technique for detecting the interaction between bioreceptor immobilized on an electrode surface and the analyte by testing the electrode/electrolyte interface and following the change in the impedance of the electrode/solution interface.

In general, a more comprehensive and complete information about the biosensing system can be obtained from EIS when compared to that one obtained from the more usual voltammetric techniques. EIS can distinguish between two or more electrochemical reactions occurring at the same time and can identify diffusion-limited reactions.

2.2 Electrode materials

The transducer is the most important component of a biosensor because it directly affects the sensor performances such as sensitivity and response time. The chemical reaction occurring in the sensing layer near the electrodic surface is transformed into an electrochemical signal. The rate and the quality of signal production is directly related to the surface properties of the electrode, the rate of electron transfer, and mass transfer. Thus, the selection of electrode material highly affects the rate of electron transfer in electrochemical biosensors. Various types of electrodes used in electrochemical biosensors are re-ported below.

The peculiar properties of gold (e.g., biocompatibility, stability, and conductivity) have promoted its use as electrodes in electrochemistry. The gold electrodes sensitivity and functionality can be enhanced modifying their surface introducing suitable molecules, and polymers.

For examples, long-chain organic compounds such as thiol have been employed for the modification of gold surfaces using self-assembling monolayers (SAM), which can be used as anchoring/immobilizing platforms of enzymes or specific bioreceptors. Such modified electrodes have been applied preferentially in several examples of biosensors.

In addition, gold nanomaterials were employed in the electrochemical bio-sensing area not only for their high conductivity, their compatibility, but also for their high surface to volume ratio. [28, 29]

Carbon has been recognized as one of the most common electrode materials used in electrochemical biosensing. The most common forms of carbon used as electrodic materials are carbon paste, glassy carbon, carbon nanotubes, and graphene electrodes, all these carbon materials are cheaper than noble metals.

Carbon paste is made of graphite powder and an organic binder, immiscible with water and is useful in insulating graphite from aqueous solutions. This kind of carbon-based electrodes presents several advantages such as low cost, low background current, regenerability, and various operating potentials. Moreover, different compounds can be easily incorporated into the carbon paste for bioanalytical applications.

Similarly, glassy carbon electrodes have also been employed for electrochemical biosensor using ad hoc modifications. However, apart from their high cost, glassy carbon electrodes need an accurate pretreatment procedure which constrains their use in many electrochemical applications.

Carbon nanotubes (CNTs) present several properties associated to their structure, functionality, morphology, and flexibility and can be classified as single-walled nanotubes (SWNTs), double-walled nanotubes (DWNTs), and multi-walled nanotubes (MWNTs) depending on the number of graphite layers. [30] Functionalized CNTs have been used in several application fields. The chemical functionalities for their application in biosensing can easily be designed and tuned through the tubular structure modification.

Graphene is one of most applied nanomaterial in the sensing area. Different graphene-based materials have been produced (e.g., electrochemically, and chemically modified graphene) using many procedures [31]. Graphene shows properties such as high conductivity, speeding up electron transfer, and a large surface area, very similar indeed to the corresponding properties of CNTS, so it is considered a good candidate for assembling sensors to determine several target molecules.

Graphene oxide (GO) is hydrophilic and can be dispersed in water solution because of the presence of hydrophilic functional groups (OH, COOH and epoxides). On the other hand, GO has a low conductivity in comparison to graphene, so reduced GO (rGO) is more employed as electrode modifier in electrochemical biosensing area [31].

In addition, non-conventional sensing platforms, such as paper and/or screen-printed electrodes (SPE), frequently modified with different nanomaterials and/or nanostructures are employed in assembling electrochemical biosensors.

Screen-printing technology offers several advantages for assembling electrochemical biosensors, including a wide range of geometries, mass production, disposability, and portability. [32] These properties are very important for commercializing biosensors.

Recent developments in fabrication of screen-printing electrodes (SPEs) were the topic not only of numerous original research papers, but also of interesting reviews, [33-34] analyzing the selection of support material, ink composition, and methods of surface modification or functionalization. Finally, in all the above-mentioned reviews, methods of obtaining of well-defined geometries and microelectrode arrays are discussed and compared for assembling smart electrochemical biosensors.

3. Application of electrochemical biosensors in food analysis

This review focused on the electrochemical biosensors as smart analytical tools for the detection of some of the most important bacteria, toxins, pesticides, antibiotics, and contaminants in foods.

3.1 Toxins

Toxins are present in a natural environment, and they are produced by microbes and algae. According to their origin, toxins are commonly classified into bacterial toxins, fungal toxins, and algal toxins. [35] Toxins contamination is unforeseeable and inevitable. In fact, it can take place during the food production chain including processing, transport, and storage, so causing severe economic losses and public health problems. Based on the survey from the World Health Organization (WHO), humans are exposed to toxins through the ingestion of contaminated foods, causing severe poisoning. [36, 37]

Herein, this review investigates the state-of-art of the electrochemical biosensors for the detection of toxins with a particular focus on several typical toxins, such as shellfish toxins, algae toxins, and mycotoxins and table 1 summarizes the analytical characteristics of recent electrochemical biosensors for toxins reported in the review

Table 1. An overview of recent electrochemical biosensors for toxins determination.

Electrode	(Bio)sensor format	Electrochemical technique	Analyte/Sample	L-R.	LOD	References
SPCEs	Label-free electrochemical aptasensor based on DNA nanotetrahedron and DNA triplex	SWV	Saxitoxin/Sea water	1–400 nM	0.92 nM	[40]
CB-SPCEs	Enzyme-linked immunomagnetic electrochemical (ELIME) assay	CA	DA/Shellfish scallop	5-62 $ngmL^{-1}$	0.4 ng mL^{-1}	[50]
Phosphorene-gold-SPCE (BP-Au.SPCE)	Electrochemical microfluidic biochip including BP-SPCE with an OA aptamer	DPV	OA/Mussels	10-250 nM	8 pM	[52]
Indium-Tin Oxide electrode (ITO)	Electrochemiluminescence (ECL) aptasensor supported by magnetic graphene oxide (M-GO	ECL/CV	OA/Mussels	0.01-10 $ngml^{-1}$	$4pgml^{-1}$	[54]
AuSPE	Electrochemical aptasensor based on aptamer-complementary strands of aptamer complex forming a π-shape structure on the surface of electrode and exonuclease I (Exo I).	DPV	AFB1/ human serum, grape juice	7–500 $pgmL^{-1}$	$2pgmL^{-1}$	[61]
AuSPE	Electrochemical immunosensor utilizing a competitive assay format	DPV	OTA and AFM1/red wine, milk	-	OTA 15$ngml^{-1}$ AFM1 37$ngml^{-1}$	[62]
SPCE	Magnetically assembled aptasensor for label-free determination of AFB1 employing a disposable screen-printed carbon electrode (SPCE) covered with polydimethylsiloxane (PDMS) film as the micro electrolytic cell.	EIS	AFB1/peanuts	20-50 $pgml^{-1}$	$15pgml^{-1}$	[63]

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GO-PAA	Aptasensor employing PAA modified with GO and an aptamer of AFB1.	Amperometry	AFB1/no real samples	1–20 ngmL-1	0.13ngmL^{-1}	[64]
SPCEs	Aptasensor using a competitive format and modified screen-printed electrode	DPV	AFB1/ maize flour	Dose-response curve 0.1-10 ngml^{-1}	0.086ngmL^{-1}	[65]
AuE	Aptasensor having methylene blu (MB) as redox tag	SWV	AFB1/white wine	2 nM–4 µM	2nM	[66]
Screen Printed Bipolar Electrode (BPE)	BPE-ECL aptasensor	ECL	AFB1/ Rice, wheat, corn, sorghum, barley, and buckwheat grains	0.1-100 ng mL^{-1}	0.033 ngmL^{-1}	[67]
GCE	Biosensor for AFB1 and ZEN using Escherichia coli as biorecognition element	CA	AFB1 and ZEN/ peanut and corn oil	AFB1 0.01–0.3 µgmL^{-1} ZEN 0.05–0.5 µgmL^{-1}	AFB1 1ngml^{-1} ZEN 6ngml^{-1}	[68]
AuE	Immunosensor based on DNA tetrahedron-structured probe (DTP), obtained from the conjugation between DNA tetrahedron nanostructures and HRP -labelled AFB1 monoclonal antibody	DPV	AFB1/ Rice, wheat, corn, sorghum, barley, and buckwheat grains	0.05-20 ngmL^{-1}.	0.033 ngmL^{-1}	[69]
LbL-GCE	Aptasensor assembled via layer-by-layer deposition of differently charged layers onto GCE. TheAFB1 aptamer was immobilized onto the negatively charged layer	EIS	AFB1/oil and soy sauce	0.001-0.10 ngmL^{-1}	0.002ngmL^{-1}	[70]
AuNPs-GO-PABA-GCE	Immunosensor where AFB1 antibodies are linked to AuNPs-GO-PABA nanocomposite, deposited on GCE	EIS	AFB1/vegetable oils	0.01-1.0 ngmL^{-1}; 1-25 ngml^{-1}	0.001ngmL^{-1}	[71]
AuE	Aptasensor where AFB1 aptamer is immobilized onto MCH layer self-assembled on AuE.	SWV	AFB1/wine, milk, corn flour	8 pM–25 nM; 25 nM-3 µM	6 pM	[72]
MBs-SPCEs	Electrochemical magnetoimmunosensor involving magnetic beads (MBs) and disposable carbon screen- printed electrodes (SPCEs)	Amperometry	FB1/beer	Non-linear calibration curves performed	0.33µg L^{-1}	[75]
AuNPs-PPy-rGO-SPCEs	Immunosensor using AuNPs-PPy-rGO nanocomposite as platform for immobilizing anti-toxin antibody	DPV	FB1, DON/corn	FB1 0.2-4.5 ppm; DON 0.05-1ppm	FB1 4.2ppb; DON 8.6ppb	[76]

NanoMIPs-PPY-ZnP-Pt	Chemosensor based on nano imprinted polymer nanoparticles (nanoMIPs) immobilized.	DPV, EIS	FB1/maize flour	1 fM –10pM	EIS 0.7 fM; DPV 0.03 fM	[77]
SPCE	Label-free electrochemical impedimetric aptasensor based on the diazonium-coupling reaction mechanism for the immobilization of anti-OTA aptamer at SPCEs	EIS	OTA/cocoa beans	0.15-2.5 $ngmL^{-1}$	0.15 $ngmL^{-1}$	[79]
AuE	Aptasensor based on modified gold electrode with conductive polypyrrole layer covalently bound to polyamidoamine dendrimers of the fourth generation (PAMAM G4), where the OTA aptamer was immobilized.	EIS	OTA/wine	-	2 ngL^{-1}	[80]
SPCE	Competitive aptasensor where biotin labelled, and free OTA competed to bind with immobilized aptamer onto the surface of a screen-printed carbon electrode (SPCE)	DPV	OTA/cocoa beans	0.15-5 $ngmL^{-1}$	0.07 $ngmL^{-1}$	[81]
Au thin-film single-electrodes	Impedimetric label free immunosensor using two antibody immobilization methods (oriented including Protein A/G and not oriented)	EIS	OTA/cocoa beans	Oriented 0.01-5 $ngml^{-1}$ Not oriented $5x10^{-3}$-0.05 $ngml^{-1}$	Oriented 0.01 $ngml^{-1}$ Not oriented $5x10^{-3}ngml^{-1}$	[82]
Bismuth coated glassy carbon electrode (BFE)	Aptasensor assembled by combining nanocomposites of gold nanoparticles (AuNPs)functionalized silica-coated iron oxide magnetic nanoparticles ($mSiO_2$@Au) and cadmium telluride quantum dots (CdTe QDs) modified graphene/AuNPs nanocomposites (AuNPs/CdTe).	SWV	OTA/ no real samples	0.2-4 $ngmL^{-1}$	0.07 $pgmL^{-1}$	[83]
AuE	Label-free electrochemical OTA aptasensor based on the peroxidase-like activity of g-C_3N_4 nanosheet (g-CNNS) and its high affinity toward single-strand DNA	CV	OTA/ red wines, juices, corns	0.2-500 nM	0.073 nM,	[84]
AuE	Signal-on electrochemical aptasensor for OTA assay based on DNA controlled layer-by-layer assembly of dual gold nanoparticle (AuNP) conjugates	DPV	OTA/wine	0.001-500ppb	0.001ppb	[85]
TGA-AuE	Electrochemical immunosensor based on self-assembling a 2-mercaptoacetic (TGA) monolayer on the surface of Au electrode to form the Au/TGA/bovine serum albumin (BSA)-OTA/anti-OTA monoclonal antibody composite probe	DPV	OTA/malt	0.1-1.0$ngmL^{-1}$	0.08$ngmL^{-1}$	[86]

Most shellfish toxins are small molecules, usually produced by toxic algae and accumulated in shellfish [38].

Wu et al. reported an overview of the different and widely used approaches in biosensing for shellfish toxins detection [39], emphasizing the importance of electrochemical biosensors and of the impedimetric ones.

Herein, some interesting examples of innovative approaches to determine shellfish toxins such as saxitoxin (STX), domoic acid (DA), and okadaic acid (OA)are reported.

Wang and coworkers reported a label-free electrochemical aptasensor assembled with nanotetrahedron and aptamer-triplex for sensitive detection of saxitoxin [40].

The aptamer technology, DNA nanotetrahedron, DNA triplex, and electrochemistry were combined for the first time to construct a label-free electrochemical aptasensor for a sensitive detection of small molecules.

A typical small molecule, saxitoxin was chosen as a model target, considering its low molecular weight and high toxicity. STX is one of the major toxins of Paralytic Shellfish Poison (PSP), and can cause shock, asphyxia and even death to fisheries and human [41].

Some concepts such aptasensors, DNA nanotetrahedron must be introduced.

Aptamers are binding oligonucleotides molecules generated by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), showing high affinity and high selectivity towards their specific molecular targets. Aptamers have attracted particular attention, especially in the research areas targeting small molecules, owing to aptamers advantages such as in vitro selection, rapid chemical synthesis, and easy chemical modification [42]. A lot of aptamers showing high affinity and selectivity vs. small molecules have been selected, such as aptamers towards marine toxins [43], mycotoxins [44], pesticides [45], etc. Various aptasensors were developed in the past decades [46]. Among them, the electrochemical aptasensors can involve easy handling and rapid response [47, 48], allowing a direct capture of the molecule target. However, the applicability of the electrochemical aptasensors towards small molecules is limited [49] and it is still under investigation.

To overcome the limitations of electrochemical aptasensors for small molecules, the aptamer and DNA triplex were combined and assembled with the nanotetrahedron to form one DNA structure, followed by immobilization on the surface of screen-printed electrodes [40]

Nanotetrahedron (NTH), a rigid DNA nanostructure assembled by four single-stranded monomers, is a spacer for the orientated immobilization of DNAs on surfaces. With the assistance of nanotetrahedron, the absorption of the immobilized DNAs was eliminated and the targets access to the immobilized DNAs was facilitated.

DNA triplex is formed by a third DNA strand composed of homopurine or homopyrimidine bonded to a DNA duplex.

The nanotetrahedron assisted the orientated immobilization of the aptamer-triplex on the surface of screen-printed electrodes, protecting the aptamer-triplex from absorption and assisting the aptamer to show full accessibility to STX. The developed aptasensor provided high sensitivity with a LOD of 0.92 nM and showed good applicability for the detection of STX in real sea-water samples, with a recovery ranging from 94.4%to 111%, good selectivity, stability, and repeatability. The authors suggested this kind of aptasensor for detection of small molecules, but application and validation on real food samples should be highly recommended.

Nelis et al. proposed an enzyme-linked immunomagnetic electrochemical (ELIME) assay for the detection of domoic acid (DA) as model target, utilizing Screen printed Carbon Electrodes (SPCEs), modifying with carbon black (CB) [50].

We remind that domoic acid (DA) is a marine toxin, produced by phytoplankton species, Nitzschia pungens, and the main toxic agent associated with incidents of Amnesic Shellfish Poisoning (ASP) on the east and west coasts of North America [51].

A comparison with SPCE pretreated by anodization (pre-SPCEs), and with SPCEs modified with other nanomaterials such as gold nanospheres (GNS) and gold nanostars (GNST) was performed.

A competitive chronoamperometric immunosensor for the domoic acid (DA) was assembled using the differently modified SPCEs. Hapten-functionalized magnetic beads were used to avoid the individual SPCEs functionalization with antibody. By a comparison among the different modified electrodes, CB-SPCE biosensor exhibited the best the electroanalytical performances. DA was determined with a detection limit that is tenfold lower compared to pre-SPCE (4 vs. 0.4 ng mL^{-1}). These results show very good agreement with HPLC data when analyzing contaminated scallops.

The method applied for detecting DA, using CB-SPCEs showed a great potential for anti-body-based determination of small molecules in a complex matrix. The ease-of-preparation of CB-SPE and the cost-effectiveness make CB an interesting and promising

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nanomaterial in comparison with carbon nanotubes and graphene for an efficient and sensitive portable nano-biosensors development.

Another known marine biotoxin produced by various dinoflagellates is okadaic acid. Chemically, OA is a polyether fatty acid derivative and exists in seafood such as shellfish. The consumption of contaminated shellfish with OA leads to diarrheic shellfish poisoning (DSP), which results in the inhibition of protein phosphatase enzymes in humans.

Singh and coworkers have described the performances of an electrochemical microfluidic biochip for the detection of OA. [52]

The screen-printed carbon electrode (SPCE) was modified by phosphorene-gold (BP-Au) nanocomposite and an aptamer specific to OA was immobilized on it.

BP-Au nanocomposites were synthesized by an in-situ, one-step method without the use of a reducing agent.

To improve the performances, a microfluidic platform was realized. The integrated system consisted of a microfluidic chip housing an aptamer modified SPCE, as a single detection module for okadaic acid. The nanomaterials and the microfluidic channels prepared were spectroscopically and electrochemically analyzed. A detection limit of 8 pM, and a linear range between 10 nM-250 nM were obtained. Selectivity studies were also performed with mussel samples in the presence of interfering species. The aptasensor did not show any cross-reactivity with other types of food toxins.

Singh et al. developed a naphthalimide-gold-based nanocomposite for the detection of okadaic acid. [53]

The composite shows a ratiometric response in the UV-Vis absorption spectrum and quenching in the fluorescence profile with a detection limit of 20 nM for OA in aqueous medium. In cyclic voltammetry, a shift was observed in the cathodic peak (-0.532 V to -0.618 V) as well as in the anodic peak (-0.815 V to -0.847 V) after the addition of okadaic acid. The developed sensor maintains its sensing ability in the pH range of 5-9 and in high salt concentrations. The developed sensor has been used for the OA detection of in water samples.

As the most recent example of detection of OA, we introduce an aptasensor developed by Lin [54].

A magnetic graphene oxide (M-GO) assisted homogeneous electrochemiluminescence (ECL) aptasensor was developed for a sensitive detection of okadaic acid (OA). The aptamer and $Ru(bpy)_3^{2+}$ were adsorbed in M-GO to prepare the ECL probe. The principle of M-GO assisted homogeneous ECL aptasensor is illustrated in Figure 1.

Figure 1. Schematic diagram of the M-GO assisted homogeneous ECL aptasensor for OA determination. Reprinted with permission from [54] Copyright 2021 Elsevier.

When OA disassociated aptamer from M-GO, $Ru(bpy)_3^{2+}$ was proportionally released from M-GO to generate the ECL signal. With the cooperation of deoxyribonuclease I (DNase I), the cyclic dissociation and degradation of aptamers induced much more available $Ru(bpy)_3^{2+}$ for signal amplification. On the other hand, the unreleased $Ru(bpy)_3^{2+}$ were still adsorbed in M-GO and magnetically separated. So, the background signal decreased, and the sensitivity was further improved. Results showed that the ECL intensity enhanced with the increasing logarithmic concentration of OA in the range of 0.01-10.0 ng mL^{-1}, and the limit of detection was 4 pg mL^{-1}.

The aptasensor has been used for OA detection in real sample of mussels and represents a cost-effective approach for a sensitive detection of marine toxins.

The most common and abundant toxins present in nature are mycotoxins. They are produced by fungi [55] and can contaminate crops and foods, inducing teratogenic, mutagenic, carcinogenic, immunosuppressive, and endocrine-disrupting effects on humans and animals. To ensure food safety and prevent contamination risks in agro-food sector, authorized levels for the most common mycotoxins in foods were established by the European Commission [56]. Therefore, a variety of electrochemical biosensors using different analyzing techniques have been developed for mycotoxins monitoring at the required concentrations.

Zhang et al. [57] reviewed the newly released mycotoxin aptasensors, with the aim to provide indications concerning practical applications and tailored design of aptasensors for mycotoxins and other analytes.

More recently, Kong [58] reported the recent advances of different new immunosensors for mycotoxin determination over the past five years. The real application possibility, the advantages, and drawbacks, together with current challenges and future perspectives of these mycotoxin immunosensors are evidenced.

Among the 400 different mycotoxins identified, aflatoxins presented high toxicity and carcinogenicity and they are responsible for around 25% of animal mortality [55].

You et al. [59] reviewed the recent advances in electrochemical biosensors for aflatoxins detection emphasizing the innovative sensing strategies based on electrochemistry, photoelectrochemistry, and electrochemiluminescence.

In the present review, some interesting examples of novel approaches and strategies to determine aflatoxins are reported and discussed.

Aflatoxins are detected in corn, peanuts, cottonseeds, nuts, almonds, figs, pistachios, spices, milk, and cheese and in a variety of other food and beverages; they are stable at high temperatures and consequently may resist to the cooking processes [55]. Four types of aflatoxins have been identified: AFB1, AFB2, AFG1, AFG2, plus two additional metabolites: AFM1 and AFM2, being AFB1 classified as the most abundant and toxic.

Among these, aflatoxin B1 (AFB1) is highly toxic, carcinogenic, mutagenic, genotoxic, and immunosuppressive, and classified as Group1 carcinogen by International Agency for Research on Cancer (IARC) [60].

An innovative electrochemical sensing strategy [61] was developed for detection of AFB1 using aptamer (Apt)-complementary strands of aptamer (CSs) complex, and exonuclease I (Exo I). A π-shape structure is organized on the surface of electrode. The presence of π-shape structure as a double-layer physical barrier allowed detection of AFB1 with high sensitivity. In the absence of AFB1, the π-shape structure remained intact, so only a weak peak current was recorded. Upon the addition of AFB1, the π-shape structure collapsed, and a strong current was recorded following the addition of Exo I. Under optimal conditions, a linear range between 7 and 500 pg mL^{-1} and a limit of detection of 2 pg mL^{-1} were observed. The developed aptasensor was also used to analyze AFB1 in spiked human serum and grape juice samples and the recoveries were 95.4-108.1%.

Another strategy based on a competitive immunoassay using a secondary antibody conjugated with alkaline phosphatase enzyme as a tag was applied for the voltammetric detection of mycotoxins, ochratoxin (OTA) and AFM1, metabolite of AFB1, using

modified gold screen printed electrodes (AuSPEs) [62]. The biosensor was validated in red wine and milk samples with no need for pre-treatment or preconcentration of the sample. The analytical signal was proportional to the toxin concentration in a wide linear range, showing a good limit of the detection at ng mL^{-1} level.

A magnetically assembled aptasensor [63] has been designed for label-free determination of AFB1 by employing a disposable screen-printed carbon electrode (SPCE) covered with a polydimethylsiloxane (PDMS) film as micro electrolytic cell. The resulting label-free aptasensor has been developed using electrochemical impedance spectroscopy as electroanalytical technique after the biorecognition between aptamers and the targets. The aptasensor showed a linear range from 20 pg mL^{-1} to 50 ng mL^{-1} with a detection limit of 15 pg mL^{-1} and was applied to detect AFB1 in spiked samples of peanuts. This sensing strategy seems to be a promising approach also for determining other targets.

An interesting AFB1 biosensor [64] is assembled by using a porous anodized alumina membrane modified with graphene oxide and an aptamer of AFB1. Briefly, the aptamer is immobilized on the surface of the porous anodized alumina nanochannels by covalent bonding. Graphene oxide is then immobilized on the surface by π-π stacking with the aptamer. On the addition of AFB1, graphene oxide is detached from the alumina surface because the specific binding between AFB1 and the aptamer, resulting in an increased current response. The increase in current is proportional to the concentration of AB1. The detection limit of the aptasensor is about 0.13 ngmL^{-1} and the linear range is from 1 to 20 ng mL^{-1}. Furthermore, a good selectivity towards AFB1 was observed, but the application to real food samples should be important for an effective sensor validation.

An electrochemical enzyme-linked oligonucleotide sensor for a rapid detection of aflatoxin B1 (AFB1) is developed by Marrazza and coworkers [65].

The assay is based on a competitive format and disposable screen-printed cells (SPCs). Aflatoxin B1 conjugated with bovine serum albumin (AFB1-BSA) was immobilized by covalent binding on electropolymerized poly (aniline-anthranilic acid) copolymer (PANI-PAA). After performing the affinity reaction between AFB1 and the biotinylated DNA-aptamer, the solution was dropped on the modified SPCs and the competition occurred. The biotinylated complexes formed onto the sensor surface were coupled with a streptavidin-alkaline phosphatase conjugate. 1-naphthyl phosphate was used as enzymatic substrate and the electroactive product was detected by differential pulse voltammetry (DPV). A dose-response curve was obtained between 0.1 ng mL^{-1} and 10 ng mL^{-1} and a limit of detection of 0.086 ng mL^{-1} was achieved. Finally, the sensor was applied for detecting AFB1 in maize flour samples.

Another electrochemical aptasensor achieving rapid detection of aflatoxin B1 (AFB1) was designed and developed by Zhao [66]. A short anti-AFB1 aptamer having a methylene blue (MB) as redox tag was immobilized on the surface of a gold electrode. Under optimized conditions, a AFB1 dynamic concentration range from 2 nM to 4 μM was obtained. The sensor could be well regenerated and reused. This sensor was able to detect AFB1 spiked in 20-fold diluted beer and 50-fold diluted white wine, respectively.

An electrochemiluminescence (ECL) platform based on a screen-printed bipolar electrode (BPE) was developed by Chen et al. [67] for a sensitive detection of aflatoxin B1 in cereals.

The sensor included a cathode of closed BPE as a sensing interface and an anode as a signal collection interface.

After mixing the test sample with a known concentration of horseradish peroxidase labeled AFB1 (HRP-AFB1), a competition for binding to monoclonal antibodies occurred. The sensor showed a good analytical performance for AFB1 with a linear range from 0.1 to 100 ng mL^{-1} and a detection limit of 0.033 ng mL^{-1}. The sensor avoids the direct contact between the reaction system and the signal measurement system. Different kind of cereals (rice, wheat, corn, sorghum, barley, and buckwheat) were selected as model grains to be tested. The results demonstrated that the recovery rate and accuracy of this sensor are at least comparable with those from ELISA.

A peculiar and innovative biosensor for the toxicity assessment of AFB1 and zearalenone (ZEN), another mycotoxin was fabricated by Ghao et al. [68].

It combines the advantages of both the electrochemical method and the peculiar characteristics of bacteria (E. coli) as the biorecognition element. The toxicity of mycotoxin AFB1 and ZEN are evaluated by the inhibition of E. coli metabolic activity. The combined toxic effect of the two mycotoxins has been investigated, and synergistic biotoxicity has been observed.

Under optimized experimental conditions, a linear concentrations range of AFB1 and ZEN in the range of 0.01-0.3 and 0.05-0.5 μgmL^{-1}, with detection limits of 1 and 6 $ngmL^{-1}$, respectively.

The recovery experiments in real oil samples (peanut and corn oils) indicated that the biosensor is applicable for the real sample mycotoxin detection.

An interesting strategy for AFB1 detection in grains [69] was based on DNA nanotetrahedron-structured probe (DTP) and horseradish peroxidase (HRP) triggered polyaniline (PANI) deposition. Briefly, the DNA nanotetrahedron was assembled on a gold electrode. Its carboxylic group was conjugated with the AFB1 monoclonal antibody (mAb) to form DTP. The test sample and a known set concentration of HRP-labeled AFB1 were mixed, and they compete for binding to DTP. The HRP assembled on the gold electrode catalyzed the polymerization of aniline on DTP. AFB1 in grains could be determined by using PANI which could be detected by using the electrochemical method. The dynamic AFB1 concentrations range was from 0.05 to 20 ng mL^{-1}. The detection limit was 0.033 ng mL^{-1}. Rice, wheat, corn, sorghum, barley, and buckwheat were selected as model grains to be tested. The results showed that the recovery rate and accuracy of this sensor are comparable with those of ELISA.

Layer-by-layer self-assembly technology was used to assemble an electrochemical EIS aptasensor for the detection of AFB1 [70]. A multi-layered sandwich structured electrode was obtained depositing alternately positively charge layers (modified graphene nanosheets) and negatively charge layers (carboxylated polystyrene nanospheres). In this way many electrochemical active sites and high conductivity were produced. The aptamer of AFB1 was immobilized on the positively charged layer via an amide bond. The optimized electrochemical aptasensor showed a limit of detection of 0.002 ng mL^{-1} and a good stability after 30 days. The electrochemical aptasensor was applied for the detection of AFB1 in oil and soy sauce, yielding recovery values in the range of 94.5 and 103.3%.

A glassy carbon electrode (GCE) modified with a nanocomposite composed by poly (4-aminobenzoic acid) (PABA), graphene oxide (GO), and gold nanoparticles (AuNps) was used for detecting AFB1 [71]. The carboxyl groups are used to bind covalently AFB1 antibodies, via self-assembly of the antibody on AuNPs surface, enhancing the binding sites for capture probe molecule and electrochemical signal. The obtained immunosensor showed good linear range from 0.01 to 1 ng mL^{-1} and from 1 to 25 ng mL^{-1}, and its detection limit is determined to be 0.001 ng mL^{-1}. This immunosensor also demonstrated satisfactory reproducibility, selectivity, and stability. Moreover, the immunosensor was able to detect AFB1 in vegetable oil samples.

An electrochemical sensor based on a modified gold electrode for detection of aflatoxin B1 (AFB1) [72], was assembled, by using a 26-mer DNA aptamer with methylene blu (MB) label on an internal thymine (T) site (e.g., 18th T) and a thiol moiety at 5′ terminal. This sensor showed a detection limit of 6 pM, and enabled detection of AFB1 in wine, milk, and corn flour samples. This sensor can be regenerated and shows good stability.

Fusarium mycotoxins are a general term for indicating the secondary metabolites produced by Fusarium species and fumonisins is one the most representative family of this kind of mycotoxins.

Approximately 15 different derivatives of fumonisins have been discovered, including fumonisin A1 (FA1), FA2, FB1, FB2, FB3, FB4, FC1, FC2, FC3, FC4 and FP1 [73].

Fumonisin B1 (FB1) is the most toxic compound in this family, exhibiting hepato-, nephro-, and immunotoxicity in many animal species. It is also classified as Group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer [60].

Guo [74] reviewed the advances in biosensors, chemosensors, and assays based on the classical and novel recognition elements such as antibodies, aptamers, and molecularly imprinted polymers. Application to food analysis, limits and time of detection were also analyzed and discussed.

In the following, some interesting examples of novel approaches and strategies to determine FB1 are reported and discussed. We would like to underline that few examples of sensors for determination of FB1 include, electrochemical biosensors, probably because they are limited to dedicated applications because of the instability of their bioreceptors and fabrication difficulties. In this regard, there is still much room for improving FB1 determination.

Escarpa and his group [75] developed an electrochemical magneto immunosensor involving magnetic beads and disposable screen-printed carbon electrode (SPCE) for fumonisins (FB1, FB2 and FB3). Once the immunochemical reactions took place on the magnetic beads, they were confined on the surface of SPCE, where electrochemical detection is achieved through the addition of suitable substrate and mediator for enzymatic tracer (Horseradish peroxidase, HRP). A detection limit of 0.33 μg L^{-1}, good repeatability, reproducibility, and accuracy with recovery rate of 85–96% were obtained. The magneto immunosensor was applied to fumonisin in beer samples with a good recovery rate of 87–105%.

Gunasekaran et al. [76] report an electrochemical immunosensing method for rapid and sensitive detection of two mycotoxins, fumonisin B1 (FB1) and deoxynivalenol (DON). A disposable screen-printed carbon electrode (SPCE) was used as sensing platform. The working electrode was modified by gold nanoparticles (AuNPs) and polypyrrole (PPy)-electrochemically reduced graphene oxide (ErGO) nanocomposite film. It can be considered a suitable platform for an effective anti-toxin antibody immobilization, with an enhanced conductivity, and biocompatibility.

Under optimized conditions, the limit of detection and linear range achieved for FB1 were 4.2 ppb and 0.2 to 4.5 ppm; and the corresponding values for DON were 8.6 ppb and 0.05 to 1 ppm. The immunosensor can specifically detect the two target toxins, even if present in the same sample. The sensor exhibited high sensitivity and low matrix interference when tested on spiked corn samples. Hence, this electrochemical immunosensing approach can be employed for a rapid detection of different mycotoxins present at the same in food.

As a more recent example, we would like to introduce a sensitive and selective electrochemical sensor using molecularly imprinted polymer nanoparticles (nanoMIPs) for FB1 recognition [77]. It is an electrochemical sensor, not properly a biosensor, but the detection strategy is very interesting and effective.

NanoMIPs were prepared by free-radical polymerisation using the solid-phase synthesis method. The sensor was assembled in two steps. First, a film of the conducting polypyrrole-zinc porphyrin composite was deposited on a Pt electrode by electropolymerisation. Then, nanoMIPs were covalently attached to this film. Both electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) were used for the sensor analytical characterization. The linear concentration range for FB1 was from 1 fM to 10 pM. The limit of detection was 0.03 and 0.7 fM, respectively. This electrochemical sensor showed no cross reactivity vs. other mycotoxins. The FB1 recovery considering the FB1 spiked maize analysis samples was between 96 and 102 %.

The last mycotoxins family we considered is that of Ochratoxins, secondary metabolites secreted by fungi species (e.g., Aspergillus and Penicillium) during their growth. They are present in different crops and beverages including coffee, wine, grape juice, and dried fruits [78]. Among them, ochratoxin A (OTA) is classified as a possible

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carcinogen by the International Agency for Research on Cancer (IARC) due to its severe toxicity [60]. In addition, OTA is chemically stable, so that it is metabolized very slowly with a half-life of more than 30 days in the body. With the recognition of its serious threat, the development of smart sensing platforms for OTA plays a crucial role in food safety.

In a recent review [78], Wang reported an overview of the conventional and novel methods of OTA detection. The latest research progress and related applications of novel OTA electrochemical biosensors are mainly described with a new perspective. Furthermore, a summary of the current limitations and future challenges in OTA analysis is included, providing reference for the further research and applications.

Nevertheless, we reported and discussed some recent and interesting examples of OTA electrochemical detection.

As first examples, a label-free electrochemical impedimetric aptasensor for rapid detection and quantitation of OTA in cocoa beans is reported [79]. The anti-OTA aptamer was immobilized on screen-printed carbon electrodes (SPCEs) via diazonium-coupling reaction. The aptasensor exhibited a limit of detection of 0.15 ng/mL, showed good selectivity and reproducibility. The increase in electron transfer resistance was linearly proportional to the OTA concentration in the range 0.15-2.5 $ngmL^{-1}$, with a recovery percentage of 91-95%, obtained in cocoa samples. The analysis can be performed on-site employing a portable EIS set up.

Another impedimetric aptasensor able to directly detect OTA without any amplification procedure has been developed always by Marty and his group [80]. This aptasensor was assembles by coating the surface of a gold electrode with a film of polypyrrole (PPy), modified with covalently bound polyamidoamine dendrimers of the fourth generation (PAMAM G4). Finally, DNA aptamers binding specifically OTA were covalently bound to the PAMAM G4. The OTA detection was performed using electrochemical impedance spectroscopy (EIS) and the results indicated that the presence of OTA led to the modification of the electrical properties of the PPy film due to the aptamers conformational changes after the OTA specific binding. The aptasensor had a dynamic range of up to 5 mg L^{-1} of OTA and a detection limit of 2 ng L^{-1} of OTA, which is below the OTA concentration authorized in food by the European legislation. The efficient detection of OTA by this electrochemical aptasensor provides a platform that can be used for the detection of various small molecules through specific aptamer association.

Marty group proposed another sensor for Ochratoxin A (OTA) detection in cocoa beans using a competitive aptasensor and differential pulse voltammetry (DPV) [81]. In this case, biotin labeled, and free OTA competed to bind with immobilized aptamer onto the surface of a screen-printed carbon electrode (SPCE). The developed aptasensor showed a good linearity in the range 0.15–5 ng mL^{-1} with the limit of detection of 0.07 $ngmL^{-1}$. The aptasensor displayed good recovery values in the range 82.1–85%, thus, showing its efficiency for complex matrices.

An impedimetric label free immunosensor for the detection of OTA always in cocoa beans is reported by Albanese and co-workers [82].

Two antibody immobilization methods (oriented and not oriented) were compared highlighting a lower limit of detection (5 pg mL^{-1}) for the not oriented immobilization and a shorter linear range in comparison with that of the oriented immunosensors which showed linearity range from 0.01 to 5 $ngmL^{-1}$ OTA. Using Atomic Force Microscopy (AFM) clarified that the oriented immobilization created a more ordered and highly dense antibody surface.

Finally, the oriented immunosensor was used to determine OTA in spiked cocoa beans samples and the results were compared with those recorded with competitive ELISA kit. The immunosensor was sensitive to OTA levels lower than 2 μgkg^{-1}, representing the lower acceptable limit for OTA according to the European legislation for the common food products.

A sensitive electrochemical aptasensor for OTA was successfully assembled by Wang [83]. This aptasensor was prepared combining a nanocomposite of gold nanoparticles

(AuNPs)functionalized with silica-coated iron oxide magnetic nanoparticles (mSiO2@Au) with another nanocomposite including cadmium telluride quantum dots (CdTe QDs), graphene and AuNPs (GAu/CdTe). The aptasensor exhibited a linear range from 0.2 pg mL^{-1} to 4 $ngmL^{-1}$, and a detection limit of 0.07 $pgmL^{-1}$.

This work provides a novel strategy for a sensitive detection of various target molecules and would have great potential in food safety monitoring and clinical diagnosis, but no analysis on real samples has been provided.

A label-free electrochemical OTA aptasensor was realized by Yang [84], taking advantage of the intrinsic peroxidase-like activity of graphite-like carbon nitride (g-C_3N_4) nanosheet (g-CNNS) and its high affinity towards single-strand DNA.

This aptasensor did not require labeled aptamer and immobilization of g-CNNS compared with previous g-CNNS-based aptasensors. As a result, this aptasensor showed a detection limit of 0.073 nM and was employed to assay OTA in the real samples, including red wines, juices, and corns.

A sensitive signal-on electrochemical aptasensor has been proposed [85] for OTA detection, based on DNA controlled layer-by-layer assembly of dual gold nanoparticle (AuNP) conjugates.

Both qualitative and quantitative analysis of OTA were thus realized by differential pulse voltammetry (DPV) signals, with a detection limit of 0.001 ppb and a dynamic range from 0.001 to 500 ppb over 6 orders of magnitude. Moreover, the real sample analysis towards OTA spiked wine samples showed good recovery results. This sensing platform can represent a promising system for the food routine quality control.

A green electrochemical immunosensor for the detection of OTA was prepared [86] by self-assembling a 2-mercaptoacetic (TGA) monolayer on the surface of the working Au electrodes to assemble the Au/TGA/bovine serum albumin (BSA)-OTA/anti-OTA monoclonal antibody composite probe for selective and sensitive detection of OTA. The immunosensor detection approach is based on indirect competitive principle and differential pulse voltammetry analysis.

Under the optimal conditions, the developed immunosensor showed a limit of detection of 0.08 $ngmL^{-1}$ in the range of 0.1 and 1.0 $ngmL^{-1}$ for OTA.

Real application in the spiked malt samples showed high accuracy with no matrix interferences for the proposed immunosensor.

3.2 Pathogenic Bacteria

Bacteria are the most common cause of foodborne diseases in the world [87]. Due to the potential treat of foodborne pathogens and the fact that the infective dose of some of them is low, pathogenic cells of some species must be totally absent from food, see for example the Salmonella case [88].

Considering all these critical issues, the development of accurate, simple, rapid, low-cost, and possibly portable devices able to make point-of-care analyses is mandatory. Biosensors seem to be suitable analytical tools, complying with the majority of all these requirements.

Du [89] reviewed recent developments in electrochemical biosensing technologies used to detect common foodborne pathogens, evidencing that biosensing technology is a sufficiently mature technology to be applied to the determination of pathogenic bacteria.

Riu [87] also reviewed novel electrochemical biosensors for pathogenic bacteria, providing a critical overview about the state-of-art of biosensors and, at the same time, some trends, and indications for future developments in this area.

The present review focuses on the most recent advances in electrochemical (bio)sensors for the detection of pathogenic bacteria in food. Papers published in the last 5-6 years are reviewed and table 2 summarizes the analytical characteristics of recent electrochemical biosensors for pathogenic bacteria reported in the review

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Table 2. An overview of recent electrochemical biosensors for pathogenic bacteria determination.

Electrode	(Bio)sensor format	Electrochemical technique	Analyte/Sample	L-R.	LOD	References
GCE	Electrochemical immunosensor based on high density gold nanoparticles (AuNPs), dispersed in chitosan (CHI) hydrogel, and modified glassy carbon electrode (GCE)	DPV	Salmonella/milk, water	10-10^5 $CFUml^{-1}$	5 $CFUmL^{-1}$	[93]
SPCEs	Label-free impedimetric aptasensor assembled by grafting a diazonium-supporting layer onto screen-printed carbon electrodes (SPCEs), followed by chemical immobilization of aminated-aptamer	EIS	Salmonella/apple juice	10-10^8 CFU mL^{-1}	6 $CFUmL^{-1}$	[94]
AuE	Label-free impedimetric aptasensor based on the combination of poly [pyrrole-co-3-carboxyl-pyrrole] copolymer and the Salmonella aptamer	EIS	Salmonella/apple juice	10^2-10^8 CFU mL^{-1}	3 $CFUmL^{-1}$	[95]
GCE	Electrochemical aptasensor developed using electrochemically-reduced graphene oxide-chitosan (rGO-CHI) composite deposited onto GCE	DPV	Salmonella/chicken	10-10^6 $CFUmL^{-1}$	10 $CFUmL^{-1}$	[96]
AuE	Electrochemical aptasensor developed by combining target-induced aptamer displacement on gold nanoparticles (AuNPs) deposited onto Au electrode with rolling circle amplification (RCA)	DPV	Salmonella/milk, mineral water	20-20^7 $CFUmL^{-1}$	16 $CFUmL^{-1}$	[97]
GF-GCE	Electrochemical immunosensor based on Anti-Salmonella antibody immobilized on the surface of the graphite felt electrode	OSWV	Salmonella/no real samples	-	10^5 E. coli cells mL^{-1}	[98]
BiSPCE	Immunosensor where bacterial cells were separated immunomagnetically and reacted with conjugate; labelled with an electrochemical indicator, including hyperbranched dendron molecules and heavy metal-derived quantum dots (CdTe QDs). Square-wave anodic stripping voltammetry(SWASV) employing screen-printed carbon electrodes with in-situ formed Bi(III) film (BiSPCE) was used for the detection and quantification of metal ions released from the QDs and correlated with the bacterium amount.	SWASV	Salmonella/milk	-	4 $CFUmL^{-1}$	[99]
AuIME	Electrochemical aptasensor using aptamer coated gold interdigitated microelectrode (IAuE) for target capture and impedance measurement, and antibody modified nickel nanowires (NiNWs) for target separation and impedance amplification.	EIS	Salmonella/chicken	10^2-10^6 CFU mL^{-1}	80 $CFUmL^{-1}$	[100]
AuIME	Immunosensor using multiple magnetic nanobead (MNB) nets in a ring channel for continuous-flow separation of target bacteria from the sample volume, manganese dioxide nanoflowers (MnO_2 NFs) for efficient amplification of biological signal, and an interdigitated microelectrode for measurement of impedance change	EIS	Salmonella/chicken	30-30 x10^5 $CFUml^{-1}$	19 $CFUmL^{-1}$	[101]
AuIME	Imedimetric immunosensor using rotary magnetic separation and cascade reaction	EIS	Salmonella/chicken	10-10^6 $CFUmL^{-1}$	10 $CFUmL^{-1}$	[102]

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AuE	Electrochemical genosensor based on the immobilization of complementary DNA on the gold electrode surface, which hybridizes with a pathogen specific fragment gene to make a sandwich structure	DPV	E. coli/beef	-	1.97x10^{-14} M	[106]
m-GECE (magnetic-Graphite Epoxy Composite (m-GEC) electrode	Electrochemical magneto-genosensor based on the detection of the tagged amplified DNA obtained by single-tagging PCR with a set of pathogen specific primers, followed by electrochemical magneto-genosensing on silica magnetic particles	Amperometry	E. coli /no real samples	0.03-3 ng mL^{-1}	0.05 ngmL^{-1}	[107]
AuE	Label-free impedimetric immunosensor using reduced graphene wrapped copper (II) assisted cysteine hierarchical structure (rGO-CysCu) as the sensing layer.	EIS	E. coli /water, fruit juice, milk	10-10^{8} CFUmL^{-1}	3.8 CFUmL^{-1}	[108]
GCE	ECL aptasensor based on AgBr nanoparticles (NPs) anchored on 3D nitrogen doped graphene hydrogel (3DNGH) nanocomposites for immobilizing luminol and enhancing its ECL behaviour.	ECL	E. coli/meal samples	0.5-500 CFUmL^{-1}	0.17 CFUmL^{-1}	[109]
SPCEs	Label-free impedimetric aptasensor using 3D-hierarchical nanostructured Bridged Rebar Graphene (BRG) for modifying SPCES	EIS	E. coli/ water, juice, and milk.	10^{2}-10^{6} CFUmL^{-1}	10 CFUmL^{-1}	[110]
PGE	Electrochemical immunosensor based on the PPy/AuNP/MWCNT/CHI hybrid nanocomposite modified pencil graphite electrode (PGE)	Amperometry	E. coli/ no real samples	30-30^{6} CFUmL^{-1}	30 CFUmL^{-1}	[111]
SPCEs	Electrochemical immunoassay using silica coated Fe_3O_4 magnetic nanoparticles (Fe_3O_4@SiO_2) and Au@Pt nanoparticles loaded on neutral red (NR) functionalized graphene to form composite complex rGO-NR-Au@Pt	CV	E. coli/ pork and milk	4.0×10^{3}-4.0×10^{8} CFU mL^{-1}	4.0×10^{2} CFU mL^{-1}	[112]
GF-GCE	Electrochemical immunosensor based on anti-Escherichia coli antibody immobilized on the surface of the graphite felt electrode	OSWV	E. coli/ beef	-	400 cells ml^{-1}	[113]
AuIME	Electrochemical biosensor based on Hybridization Chain Reaction (HCR)	MSPQC	S. aureus/milk and human serum	50-10^{7} CFUmL^{-1}	50 CFUmL^{-1}	[115]
AuE	Electrochemical biosensor based on a triple-helix molecular switch which can control the switching of electrochemical signals	DPV	S. aureus/water and honey	30-30 x10^{8} CFUml^{-1}	8CFUmL^{-1}	[116]
AuE	Label-free impedimetric immunosensor based on bacteria-imprinted conductive poly(3-thiopheneacetic acid) (BICP) film	EIS	S. aureus/milk	10-10 x10^{8} CFUml^{-1}	2CFUmL^{-1}	[117]
AuE	Dual signal amplification electrochemical biosensor based on a DNA walker and DNA nanoflowers	DPV	S. aureus/water and honey	60-60 x10^{7} CFUml^{-1}	9CFUmL^{-1}	[118]
SPCNF/AuNPsE	*plcA*-based electrochemical DNA biosensor using screen printed CNF/AuNps electrode.	CV	L. monocytogenes/milk	0–0.234 ng/6 µl	82 fg/6 µL	[128]
Pt-IME	Aptasensor using platinum interdigitated microelectrodes (Pt-IME) biofunctionalized with Listeria-specific aptamer and a smartphone-based signal acquisition system	EIS	L. monocytogenes/ vegetable broth, hydroponic media	10^{2}-10^{6} CFUmL^{-1}	23 CFUmL^{-1}	[129]

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SPCEs	Electrochemical immunosensor using disposable screen-printed electrode as transducer surface and monoclonal and polyclonal antibodies specifically recognizing Listeria monocytogenes p60 protein used as the sandwich immuno-pair.	CV	L. monocytogenes/milk	5-150 ng mL^{-1}	1.5 $ngmL^{-1}$	[130]
Disposable electrical printed (DEP) microarray electrodes	Electrochemical biosensor assembled by selectively functionalizing the array electrodes with bacteria specific peptides.	SWV	L. monocytogenes/no real samples	10-10^7 CFU mL^{-1}	9 $CFUmL^{-1}$	[131]

There are many kinds of pathogens producing toxins causing foodborne diseases [90], among them Escherichia coli, Staphylococcus aureus, Salmonella, and Listeria monocytogenes are common.

Salmonella is a species of rod-shaped Gram-negative bacteria belonging to the family of Enterobacteriaceae. It contains two main species, Salmonella enterica and Salmonella bongori with more than 2,500 serotypes and all these serotypes can cause disease in humans [91].

WHO declared Salmonella as one of the four major global causes of diarrheal diseases and one of the pathogenic bacteria with emergent resistant serotypes [92].

Considering all these criticalities, including extremely low infection limits (1 CFU), the levels of Salmonella in food regulated by laws have been tightened over the years. For example, European Commission [88] required the absence of Salmonella in a defined amount of a food product (e.g., 10 or 25 g) placed on the market during the shelf life.

Magalhães [92] has reviewed the commercially available rapid methods for Salmonella detection. The potentialities of electrochemical biosensors for the development of rapid devices are highlighted. The state-of-art and the newest and innovative technologic approaches are presented, and a critical analysis of the literature has been carried out, evidencing the current challenges towards a complete solution of the Salmonella detection criticalities.

More recently, Li [91] has presented a more general overview on Salmonella biosensors by highlighting the different typology (optical, electrochemical, piezoelectric, etc. biosensors) and analyzing recent trends, particularly the integration with nanomaterials, microfluidics, portable instruments, and smartphones.

Nevertheless, we reported and discussed some innovative and interesting examples of Salmonella electrochemical biosensors. Generally, the detection of Salmonella enterica serotype Typhimurium is considered, so, for this reason and for reasons of brevity, it will be referred to as Salmonella, unless otherwise stated.

Kraatz and co-workers [93] have developed an electrochemical immunosensor for detection of Salmonella based on glassy carbon electrode modified with high density gold nanoparticles (AuNps) well dispersed in chitosan hydrogel. The composite film has been used as a platform for the immobilization of biorecognition element such as capture anti-body (Ab1). A sandwich electrochemical immunosensor has been assembled after incubation with Salmonella and the Horseradish Peroxidase (HRP) Salmonella secondary anti-body (Ab2). The immunosensor showed a linear range from 10 to 10^5 $CFUmL^{-1}$ with a low detection limit of 5 $CFUmL^{-1}$. Furthermore, the performances of the sensor in the real-to-life conditions were tested analyzing tap water and milk samples, containing Salmonella. The results were compared and validated with those obtained by the plate counting method, indicating that the immunosensor is suitable for food safety analysis.

A label-free impedimetric aptamer-based biosensor [94] for Salmonella detection was prepared by grafting a diazonium-supporting layer onto screen-printed carbon electrodes (SPCEs), because this procedure allowed the formation of a denser aptamer layer, resulting in a higher sensitivity.

The developed aptamer-biosensor responded linearly, on a logarithm scale, over the con-centration range from $1x10^1$ to $1x10^8$ CFU mL^{-1}, with a limit of detection of 6 CFUmL^{-1}. Selectivity studies showed that the aptamer biosensor could discriminate Salmonella from six other model bacteria. Finally, the aptamer biosensor was applied to the salmonella detection in spiked apple juice samples with good recovery results.

Another label-free impedimetric biosensor [95] for the detection of Salmonella was developed based, this time, on the combination of poly [pyrrole-co-3-carboxyl-pyrrole] copolymer and aptamer. The aptamer/target interaction on the conjugated copolymer and the copolymer conductivity improved the impedimetric measurements. The aptasensor detected Salmonella in the concentration range$1x10^2$–$1x10^8$ CFU mL^{-1} with good selectivity vs. other model pathogens and with a limit of detection of 3 CFUmL^{-1}.

Finally, like the previous example [94], the aptamer biosensor was applied to the Salmonella detection in spiked apple juice samples with good recovery results.

An electrochemical aptasensor [96] was assembled employing a thiol functionalized aptamer-immobilized onto the electrochemically reduced graphene oxide-chitosan composite (rGO-CHI) as a conductive platform for the Salmonella detection.

The sensitivity and selectivity of this aptasensor against the pathogen target was evaluated using cyclic voltammetry and differential pulse voltammetry. The developed aptasensor is specific to Salmonella and can distinguish Salmonella from other pathogens. The aptasensor showed a low limit of detection of $1x10^1$ CFU mL^{-1}. The sensor was applied to artificially contaminated raw chicken samples and the results were coherent with those obtained from pure cultures.

A highly sensitive and specific electrochemical aptasensor [97] for Salmonella detection was developed by combining target-induced aptamer displacement on gold nanoparticles (AuNPs) modified gold electrode with rolling circle amplification (RCA). The sensor showed a detection limit of 16 CFU mL^{-1} and a linear detection range from 20 to 2×10^8 CFU mL^{-1} and, also demonstrate, acceptable reproducibility and low matrix effect.

The proposed strategy was further applied to some real samples for evaluating the recovery, so different concentration of Salmonella were spiked into bottled mineral water and into pure milk. The recovery results are good for both samples typologies.

A Salmonella biosensor able to process a large sample volume [98] was developed by Capobianco, by using an Ag/AgCl reference electrode, a platinum counter electrode, and a porous working electrode made from graphite felt coated with antibodies specific for Salmonella antigens. This design allows samples to flow-through the electrode while capturing target pathogens.

Detection limit of 1000 Salmonella cells was obtained in samples with a volume of 60 mL. The low cost of the sensor allows for incorporation into disposable detection devices, but an evaluation of the sensor analytical parameters on real samples, recovery included, should be very useful.

Korecka et al. [99] developed a fast and efficient biosensor for the screening of milk samples contaminated by Salmonella. A smart approach was performed where bacterial cells were separated immunomagnetically, with subsequent reaction with conjugate, i.e., specific IgG molecule labelled with an electrochemically indicator.

The peculiar structure of this indicator included hyperbranched dendron polymeric molecules and heavy metal quantum dots (QDs). Square-wave anodic stripping voltammetry (SWASV) using screen-printed carbon electrodes modified with in-situ formed Bi (III) film (BiSPCE) was used for the determining the metal ions released from the QDs (CdTe). The metal ion signals can be correlated to the number of detected bacteria cells. By this method, the Salmonella samples were analyzed in 2.5 h, even evidencing even a minimal number of bacterial cells (4 CFU) in 1 mL of the sample. The whole system was verified using real food samples. UHT whole milk samples were artificially spiked, and the obtained results are very promising.

A sensitive electrochemical aptasensor [100] was developed by Lin and co-workers using aptamer coated gold interdigitated microelectrode for target capture and

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impedance measurement, and antibody modified nickel nanowires (NiNWs) for target separation and impedance amplification.

A linear concentration range was obtained from $1x10^2$ to $1x10^6$ $CFUmL^{-1}$ in 2 h with the detection limit of 80 $CFUmL^{-1}$. The mean recovery for the spiked chicken samples was 103.2% and it can be considered acceptable.

In a more recent paper, Lin [101] designed and assembled another impedance biosensor always for detection of Salmonella using multiple magnetic nanobead (MNB) nets in a ring channel for continuous-flow separation of bacteria cell from 10 mL of sample, manganese dioxide nanoflowers (MnO_2 NFs) as nanomaterial for biological signal amplification, and an interdigitated microelectrode for sensitive impedance measurements. The approach is comparable with the other illustrated in the previous paper [100], but the role of MNB nets is new since they act as separation element of the target bacteria.

This biosensor was able to separate ~60% of Salmonella from 10 mL of bacterial sample and detect Salmonella with a linear range of 3.0×10^1 to 3.0×10^6 $CFUmL^{-1}$ in 1.5 h with lower detection limit of 19 $CFUmL^{-1}$. Moreover, this biosensor was evaluated by detecting the target bacteria in spiked chicken meat samples and the results are comparable with those obtained on the same samples with the plate counting method, indicating that it is suitable for food safety analysis.

The last impedance biosensor for Salmonella developed by the Lin group [102] is based on rotary magnetic separation and cascade reaction. First, magnetic nanoparticles (MNPs) modified with anti-Salmonella monoclonal antibodies were injected into a capillary in the presence of a rotary gradient magnetic field. Then, a bacterial sample was injected into the capillary and the target bacteria were continuous-flow captured onto the MNPs. When organic-inorganic hybrid nanoflowers were prepared using manganese dioxide (MnO_2), glucose oxidase (GOx) and anti-Salmonella polyclonal antibodies (pAbs), they were injected to label the bacteria, resulting in the formation of MNP-bacteria-nanoflower sandwich complexes. Finally, glucose (low conductivity) was injected and oxidized by GOx on the complexes to produce H_2O_2 (low conductivity) and gluconic acid (high conductivity), leading to an impedance decrease. Besides, the produced H_2O_2 triggered a cascade reduction of MnO_2 into Mn^{2+}, leading to further impedance decrease. The impedance changes were measured using an interdigitated microelectrode and correlated to the concentration of target bacteria. This biosensor was able to detect Salmonella ranging from $1x10^1$ to $1x10^6$ $CFUmL^{-1}$ in 2 h with a low detection limit of 10^1 $CFUmL^{-1}$ and a mean recovery of 100.1% for the spiked chicken samples. Considering the last three papers, the basic approach is very similar. On the other hand, a real comparison including not only the evaluation of the analytical performances, but also an evaluation of costs, specificity and reproducibility is mandatory.

In addition to Salmonella another pathogenic bacterium commonly associated with food-borne outbreaks is Escherichia coli. The infection is usually acquired via the fecal oral route by consuming contaminated and raw food, such as beef, various leaf vegetables, unpasteurized milk, and water. It should be underlined that food/water borne diseases due to E. coli is one of the major causes of illness in many developing countries [103], causing gastroenteritis and related diseases leading to dramatic consequences [104].

Recently in 2017, Li and coworkers have reviewed [105] the advancements in the development of electrochemical biosensors for the rapid detection of Escherichia coli, illustrating the different configurations of biosensors and the sensing approaches.

In this review we reported some interesting and innovative examples of E. coli electrochemical biosensors, developed in the last 5-6 years.

The electrochemical genosensor is one of the most promising methods for the rapid and reliable detection of pathogenic bacteria and an electrochemical genosensor was developed by Sun for E. coli detection [106].

The genosensor included a gold electrode where complementary DNA was immobilized, hybridizing with a specific fragment pathogen gene to build a sandwich

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structure. Multiwalled carbon nanotubes (MWCNT), embedded in chitosan with a layer of bismuth, modified a GCE for detecting the performances of the sensor.

The detection limit was 1.97 x 10^{-14} M. The genosensor showed good sensitivity and selectivity and it was also applied for determine the pathogen in real beef samples contaminated artificially.

A magneto-genosensing approach for the detection of the three most common pathogenic bacteria in food safety, such as Salmonella, Listeria and Escherichia coli is developed by Alegret group [107]. The methodology is based on the detection of the tagged amplified DNA obtained by single-tagging PCR with a set of specific primers for each pathogen, followed by electrochemical magneto-genosensing on silica magnetic particles (silica MPs). A set of primers were selected for the amplification, being one of the primers for each set tagged with fluorescein, biotin and digoxigenin coding for Salmonella enterica, Listeria monocytogenes and E. coli, respectively. The single-tagged amplicons were then immobilized on silica magnetic particles based on the nucleic acid-binding properties of silica particles in the presence of the chaotropic agent such as guanidinium thiocyanate. The assessment of the silica MPs as a platform for electrochemical magneto-genosensing is described. A linear concentration range from 0.03 to 3 ngmL^{-1} was observed with detection limits of 0.04, 0.13 and 0.05 ng mL^{-1} for S. enterica, L. monocytogenes and E. coli, respectively. It should be noticed that an evaluation of the sensor analytical parameters on real samples, recovery included, resulted very useful, at least for one of the three pathogens.

A graphene wrapped copper (II) assisted cysteine hierarchical structure (rGO-CysCu) has been used as sensing layer to assemble an impedimetric label-free electrochemical immunosensor for quantitative determination of Escherichia coli [108].

Under optimal conditions, the calibration plots was linear in the detection range of 10 CFU mL^{-1} to 1 x 10^{8} CFU mL^{-1} with a detection limit of 3.8 CFU mL^{-1}. Moreover, the proposed immunosensor showed good selectivity and specificity towards the non-pathogenic E. coli and other bacterial cells in the synthetic samples. The validation of the immunosensor was carried out using artificially contaminated real samples (E. coli spiked tap water, juices, and skimmed milk) and the results are comparable with those obtained by means of plate count method.

Wang has assembled [109] all-solid-state luminol-electrochemiluminescence (ECL) Escherichia coli aptasensors, by using AgBr nanoparticles/3D nitrogen-doped graphene hydrogel (AgBr/3DNGH).

The multifunctional nanoarchitecture was used as an all-solid-state ECL platform for assembling a E. coli aptasensor via glutaraldehyde as crosslinker between amine-functionalized E. coli aptamer and luminol/AgBr/3DNGH. Since E.coli can significantly decrease the ECL intensity because of the steric hindrance mechanism, the proposed aptasensor displayed a linear response for E.coli in the range from 0.5 to 500 CFUmL^{-1} with a detection limit of 0.17 CFUmL^{-1}. In figure 2 the preparation steps and detailed measurement sequence of the aptasensor is illustrated

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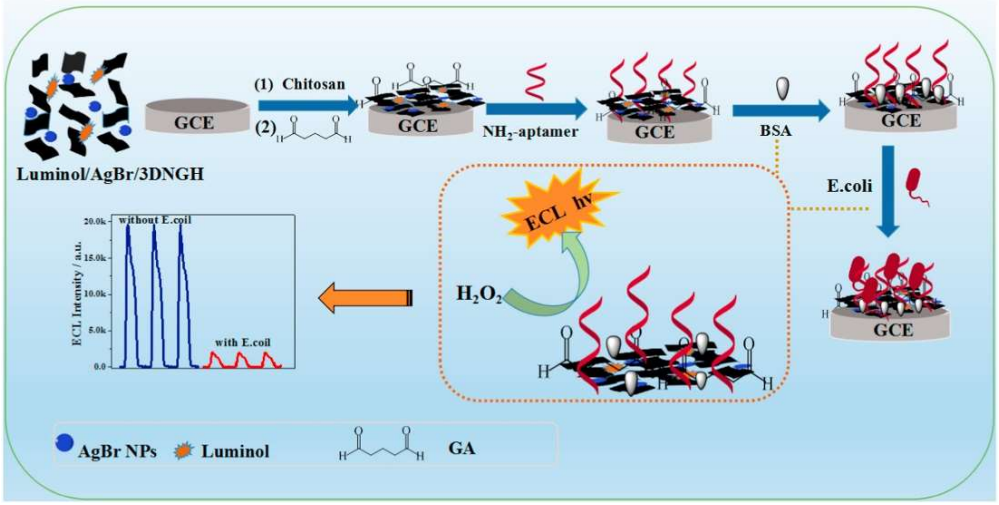

Figure 2. The schematic representation related to the ECL Escherichia coli biosensor, fabricated with luminol /AgBr/3DNGH. Reprinted with permission from [109] Copyright 2017 Elsevier.

To further demonstrate the applicability of the proposed aptasensor, the recovery test was performed by standard addition method. Different concentrations of E. coli were added into meal samples. The recovery rate ranged from 99.4% to 101.2%, indicating that the proposed method was stable and could be applied for analysis of real samples.

A Bridged Rebar Graphene (BRG) functionalized label free impedimetric aptasensor for E. coli detection was developed by Sabherwal et al. [110]. BRG was synthesized by chemical unscrolling of MWCNT for producing Graphene, followed by bridging with terephthalaldehyde (TPA) to form a 3D hierarchical nanostructure. A scheme related to the aptasensor assembling and sensing approach is illustrated in figure 3.

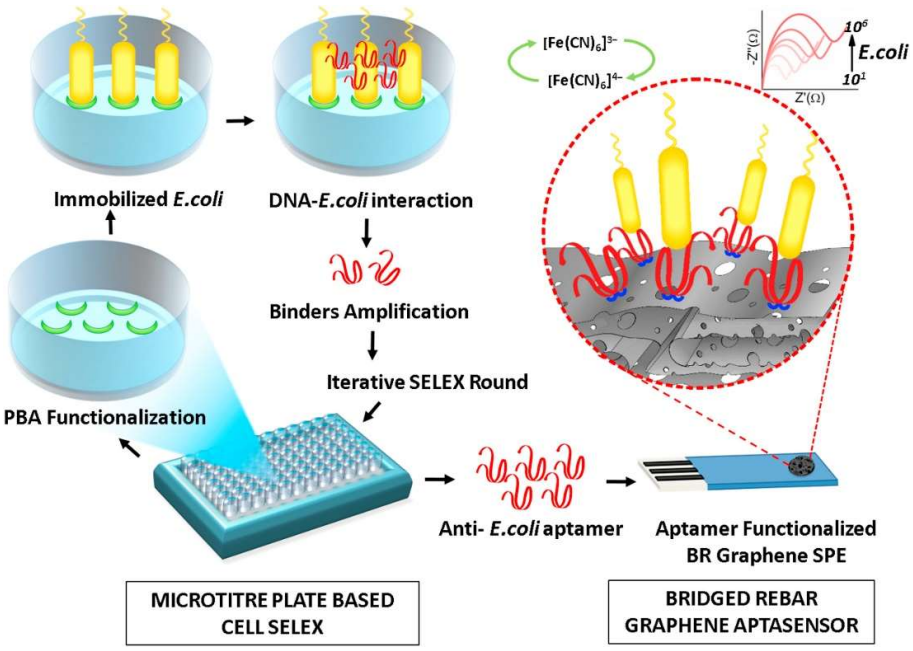

Figure 3. Schematic representation of the assembling and the sensing approach of the E. coli aptasensor. Reprinted with permission from [110] Copyright 2017 Elsevier.

The developed nanostructured aptasensor demonstrated a low detection limit of 10 CFUmL^{-1} with a dynamic response range from 10 to 1x 10^{6} CFUmL^{-1} in spiked water, juice, and milk samples.

An immunosensor based on a hybrid nanocomposite composed by poly(pyrrole), gold nanparticles, multwalled carbon nanotubes and chitosan (PPy/AuNP/MWCNT/Ch) was developed [111]. This hybrid nanocomposite modified a pencil graphite electrode (PGE) and the anti-E. coli monoclonal antibody was immobilized on the resulting platform.

Under the optimum conditions, concentrations of E. coli from 3 x 10^{1} to 3 x 10^{7} CFU/mL^{-1} were detected with a detection limit of 30 CFUmL^{-1} in PBS buffer and, also good results in terms of specificity and stability were achieved.

On the other hand, it should be mentioned that an evaluation of the sensor analytical parameters on real samples resulted very useful.

Dou [112] has reported the assembling of a non-enzymatic sandwich-type electrochemical immunoassay for quantitative monitoring of Escherichia coli. Silica coated Fe_3O_4 magnetic nanoparticles (Fe_3O_4@SiO_2)were modified with mouse anti-E. coli monoclonal antibody (Ab1) as capture probes reducing the measurement time and increasing the sensitivity. Au@Pt nanoparticles were loaded on neutral red (NR) functionalized graphene producing a nanocomposite rGO-NR-Au@Pt with high specific surface area and good biocompatibility and acting as carrier of detection antibodies (Ab2).

Under the optimized conditions, a linear concentration range is from 4.0×10^{3} to 4.0×10^{8} CFU mL^{-1} and the limit of detection is 4.5×10^{2} CFU mL^{-1}. The immunoassay exhibits acceptable specificity, reproducibility, and showed good performance in terms of recovery analyzing spiked commercial pork and milk samples.

Recently, Capobianco [113] proposed a flowthrough immunoelectrochemical biosensor for E. coli detection, taking advantage of the same sensing approach for determining Salmonella [98]. As previously reported [98], the working electrode was a porous, antibody-coated graphite felt electrode acting as both a biorecognition-element coated for capturing target pathogen as well as a signal transducer.

The low detection limit for a sample containing10,000 E. coli cells in 5, 60, and 1000 mL of buffer was 2000, 170, and 10 cells mL^{-1}, respectively in a total assay time of 3 h whereas the low detection limit for E. coli was 400 cells mL^{-1} in spiked beef samples.

Staphylococcus aureus is one of the most common foodborne pathogens and its infections can cause even more deaths than AIDS, tuberculosis and viral hepatitis combined [114].

Oh [114] reviewed the state of- the-art of biosensing approaches and methodologies for detecting S. aureus, illustrating the most used ones, based on different transducing mode, such as electrochemical, optical, and mass based biosensors.

Herein, we focused the attention on the most recent developments of the electrochemical biosensors for S. aureus determination, providing some interesting examples.

An electrochemical biosensor for rapid detection of S. aureus based on silver wire across electrodes was reported by He [115].

A multichannel series piezoelectric quartz crystal (MSPQC) was utilized as detector. For the Staphylococcus aureus determination, a linear concentration range from 50 to 10^{7} CFUmL^{-1} within 100 min was obtained. The detection limit was 50 CFU mL^{-1}.

Moreover, the proposed biosensor showed good selectivity and specificity towards other bacteria and pathogens such as Escherichia coli, Salmonella enteritidis, Listeria innocua, Pseudomonas aeruginosa and Streptococcus pneumoniae. Artificially contaminated human serum samples and milk samples were analyzed with the proposed biosensor, obtaining good recovery data ranging from 89.00% to 111.33%.

An electrochemical biosensor for Staphylococcus aureus was designed, based on a triple-helix molecular switch, which can control the switching of electrochemical signals [116]. The biosensor showed a dynamic range from 30 to 3 x 10^{8} CFUmL^{-1}, with a detection

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limit of 8 CFUmL^{-1}. In addition, the sensor is used for the detection of S. aureus in spiked lake water, tap water and diluted honey samples, with acceptable recovery results. Using the biosensor particular design, the same sensing approach has been also successfully applied for Escherichia coli detection.

An impedimetric sensor, based on bacteria-imprinted conductive poly(3-thiopheneacetic acid) (BICP) film was developed for the label-free detection of S. aureus [117]. The BICP film was in-situ synthesized and deposited on gold electrode surface. To obtain the optimal sensing performance, a lot of factors affecting the imprinting and recognition steps were studied and performed.

Under the optimized conditions, a rapid recognition within 10 min, a limit of detection of 2 CFUmL^{-1}, and a linear concentration range from 10 to 10^8 CFUmL^{-1} were obtained. The sensor also showed high selectivity, and repeatability.

Furthermore, the label free impedimetric sensor was applied to the determination of S. aureus to artificially contaminated milk samples with good recovery results.

As last and significative example, we introduce a dual signal amplification electrochemical biosensor based on a DNA walker and DNA nanoflowers for the detection of S. aureus, developed by the Zhou group [118] and illustrated in figure 4.

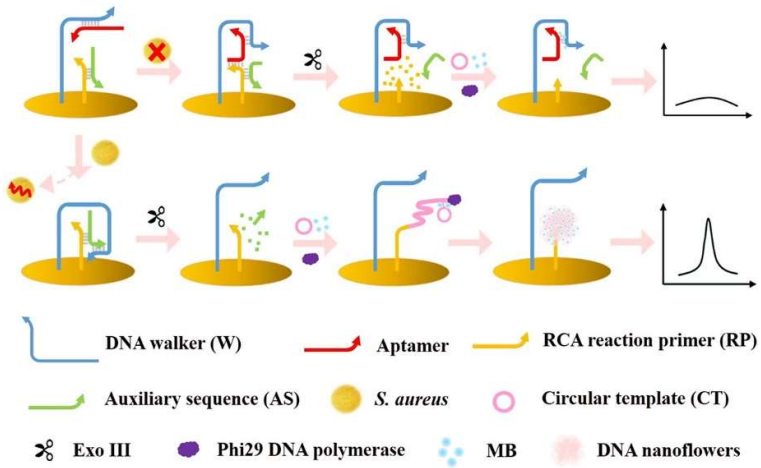

Figure 4. Schematic representation of the biosensor for S. aureus based on a DNA walker and DNA nanoflowers. Reprinted with permission from [118] Copyright 2021 American Chemical Society

Briefly, some details about DNA walker are necessary. In addition to the construction of static DNA nanomaterials, DNA can also be used to form molecular machines with dynamic behaviors [119]. Like the motor proteins responsible for the cellular movement, a DNA walker is a kind of nanoscale molecular device/nanomachine, driven by environmental stimulation, enzyme reaction, or strand displacement reaction.[120,121] It can carry out repeated mechanical cycle movement along the DNA orbit composed of nucleic acids to realize a signal cascade amplification.[122]

Two groups of double-stranded DNA are immobilized on the surface of a gold electrode. The bond of S. aureus with its aptamer caused the disintegration of the long double strands, so releasing the DNA walker. With the exonuclease III (Exo III) support, the DNA walker moves along the electrode surface, hydrolyzing the anchored short double strands. After introducing a specially customized circular DNA and phi29 DNA polymerase, the rolling circle amplification (RCA) reaction was launched. DNA nanoflowers are formed at high local concentration of DNA in the solution, so creating binding sites for the electroactive label i.e., methylene blue (MB) and thus yielding an intense signal. Under optimized conditions, the current response is linearly correlated to the logarithm of the S. aureus concentrations, ranging from 60 to 6 x 10^7 CFUmL^{-1}, and the

detection limit is 9 CFUmL^{-1}. Finally, the proposed biosensor has been applied to water samples and diluted honey samples, achieving good results in terms of recovery.

Listeria genus consists of rod-shape Gram-positive bacteria and includes seventeen different species [123]. Among them, Listeria monocytogenes (LM) is responsible to listeriosis in humans [123] and it was classified as an opportunistic, dangerous pathogen, especially for population high-risk groups such as pregnant women, children, old, and immunosuppressed people. Listeriosis could lead to serious diseases such as meningitis, fetal anomalies, abortion, febrile gastroenteritis or even generalized infection [123].

Despite the low incidence, when compared to other common foodborne diseases (e.g., Salmonellosis or Escherichia coli infections), LM infection is associated to a greater number of hospitalizations and to a higher mortality rate (20–30%) [123]. In addition, the fact that LM can grow in different food commodities (i.e., dairy products, raw and preserved animal meats and vegetable products) [124] and in adverse environments exacerbates the problem.

Soni [125] provided a general overview concerning the emerging trends for Listeria M. detection, summarizing the developments in optical, piezoelectric, cell-based, and electrochemical biosensing detection, applied in different fields such as clinical diagnostics, food analysis, and environmental monitoring, and, also, evidencing their drawbacks and advantages.

Narang and co-workers published a review [126] concerning the evolution of analytical techniques for Listeria M., highlighting the importance and the performances of the electrochemical ones.

Regarding the emerging electrochemical approaches for Listeria M. detection in food, Delerue-Matos [127] provided an accurate overview evidencing the use of low-cost electrochemical transducers, integration of new nanomaterials and incorporation of new bio-receptors in the sensing strategy.

Herein, we propose some significative and interesting approaches for LM detection in the electrochemical biosensing area.

The first *PlcA*-based nano-assembled electrochemical DNA biosensor [128] has been developed for the detection of Listeria monocytogenes in raw milk samples, using screen-printed carbon electrodes (SPCEs) modified with Graphitized Carbon Nanofibres (CNFs) and gold nanoparticles (AuNPs).

Considering that the bacterium contains different virulent factors that disrupt the vacuolar membrane, phosphatidylinositol-specific phospholipase C gene (PlcA) of L. monocytogenes is a virulent gene and encodes a 33-kDa protein responsible for the lysis of primary single-membraned vacuoles.

The selectivity of the developed biosensor was analyzed and confirmed using complementary and mismatch oligonucleotide sequences. The limit of detection was found to be 82 fg in 6 µl. The electrode was stable for six months. The validation study was performed using different milk samples artificially spiked with L. monocytogenes and the results obtained demonstrated that it can be applied for Listeria m. detection in raw milk, moreover with good specificity.

Gomes group [129] an innovative Listeria aptasensor using platinum interdigitated microelectrodes (Pt-IME). The sensor is incorporated into a particle/sediment trap for the real-time analysis of irrigation water in a hydroponic lettuce system. This system was used for rapid on-site analysis of water quality, using a smartphone-based potentiostat. In flow conditions (100 mL samples), the aptasensor showed a detection limit of 48 CFUmL^{-1} with a linear range of 10^2 to 10^4 CFU mL^{-1}. In no flow conditions, the aptasensor was applied for Listeria detection to vegetable broth, and hydroponic samples. Finally, this is the first example where an aptasensor has been used for testing microbial water quality for hydroponic lettuce in real time, using a smartphone-based acquisition system according with the standards. The aptasensor showed a good recovery of 90 %.

Delerue-Matos [130] reported the development of an electrochemical immunosensor for rapid, specific, and decentralized detection of the invasion-associated protein p60

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secreted by Listeria monocytogenes. To accomplish a more specific detection, different genes (hly, iap) and their corresponding encoded proteins, listeriolysin O and p60, considered as the major virulence factors associated with pathogenic action, have been targeted for the detection of Listeria M.

Particularly, p60 protein has an important role in host invasion, cell division and viability and besides being a cell surface protein is also secreted in large quantities into the growth media. These features make p60 an ideal diagnostic target for the development of immunological detection systems.

A disposable screen-printed electrode was used as transducer and monoclonal and polyclonal antibodies, specifically recognizing Listeria monocytogenes p60 protein and Listeria spp. p60 proteins, were used as the sandwich immunopair, so the pathogenic Listeria can be distinguished from the non-pathogenic ones. The analytical signal was acquired through the voltammetric stripping of the enzymatically deposited silver, which was directly correlated to p60 concentration in the sample. In optimized conditions, a limit of detection of 1.5 ng mL^{-1} was obtained in less than 3 h. As proof-of-concept, the proposed immunosensor was successfully applied to spiked milk samples, obtaining good results in terms of recovery.

A novel electrochemical biosensor is reported for simultaneous detection of Listeria monocytogenes and Staphylococcus aureus [131]. The biosensor is composed by a gold nanoparticles-modified screen-printed carbon electrodes on which magnetic nanoparticles coupled to specific peptides were immobilized. Taking advantage of the proteolytic activities of the protease enzymes produced from the two bacteria on the specific peptides, the detection was achieved in 1 min. Limits of detection of 9 CFU mL^{-1} for Listeria monocytogenes and 3 CFU mL^{-1} for Staphylococcus aureus were obtained. Good selectivity of the biosensor was demonstrated by analyzing samples containing at the same time Staphylococcus aureus, Listeria monocytogenes and E. coli. This platform seems to be promising for a rapid, and cost-effective simultaneous detection of various bacteria, but it should be noticed that an evaluation of the sensor analytical parameters on real samples, recovery included, resulted very useful, at least for one of the two pathogens.

As last example we introduce a not-electrochemical sensor which integrates the sensitivity of magnetic sensing and efficiency of the hybridization reaction, providing an innovative and promising detection platform for pathogens.

A magnetic DNA sensor based on nucleic acid hybridization reaction and magnetic signal readout was proposed very recently by Chen [132]. This biosensing system allows the one-step detection of L. monocytogenes as low as 50 CFUmL^{-1} within 2 h without DNA amplification, and the average recovery in the spiked ham sausage samples resulted to be 92.6%.

3.3 Pesticides

Pesticides are among the most used products in the agri-food industry for the control, prevention, and elimination of pests. According to the target pest, they can be classified in insecticides, fungicides, herbicides, etc. The main classes of pesticides are the following carbamates, organophosphates, pyrethroids, or triazines, among others [133] and all these compounds resulted highly toxic. According to the World Health Organization (WHO), they can be classified as carcinogenic, neurotoxic, or teratogenic [134].

In the European Union, the Residual Maximum Limits (MRLs) legally permitted are 0.1 μg/L for a single pesticide and 0.5 μg/L for total pesticides [135].

Therefore, for pesticides monitoring at the required MRLs, a great number of electrochemical biosensors using different analyzing techniques have been developed and some relevant examples have been summarized in table 3.

Table 3. An overview of recent electrochemical biosensors for pesticides determination.

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Electrode	(Bio)sensor format	Electrochemical technique	Analyte/Sample	L-R.	LOD	References
GCE	Electrochemical sensor using GCE modified with MCNHs and zein (SWCNH-ZE/GCE)	DPACSV	Fenitrothion/orange juice	9.9×10^{-7}-1.2×10^{-5} M	1.2×10^{-8} M.	[138]
SPCEs	Electrochemical sensor based on SCPCEs modified with cellulose microfibers supported reduced graphene oxide composite	DPV	Fenitrothion/water	0.03–1333.8 μM	8 nM	[139]
GCE	Electrochemical biosensor using glutaraldehyde (Glu) cross-linked with acetylcholinesterase (AChE) immobilized on s-SWCNTs wrapped with bovine serum albumin (BSA)	DPV	Parathion/strawberry and apple juice	$1x10^{-10}$-$5x10^{-6}$ M	$3.75x10^{-11}$ M	[140]
ITO	Electrochemical sensor using ITO electrode modified with poly 3,4-ethylenedioxythiophene (PEDOT) membrane and zirconia nanoparticles (ZrO_2 NPs)	CV	Parathion/water	5–2000 $ng{\cdot}mL^{-1}$	2.8 $ng{\cdot}mL^{-1}$	[141]
SPCEs	Electrochemical biosensor using the multi-dimensional nanocomposite (MXene/Au-Pd) as the functional platform for immobilizing AChE.	Amperometry	Paraoxon/pear and cucumber	0.1-1000 μg L^{-1},	1.75 ngL^{-1}	[142]
Fluorine -tin oxide glass electrodes (FTO)	Electrochemical sensor developed by immobilizing haemoglobin (Hb), redox active protein on electrochemically reduced graphene oxide-chitosan (ERGO-CHI/Hb/FTO)	SWV	Parathion/onion, lettuce	0.076-0.988 μM	79.77nM	[143]
GCE	Electrochemical sensor using reduced graphene oxide (RGO) decorated fumed silica (FS) to modify glassy carbon (FS@RGO-GCE).	DPV	Fenitrothion/orange juice and tomato	0.005-1.0 μM	0.00019 μM	[144]
GCE	Electrochemical sensor using silver nanoparticles /dodecane modified glassy carbon electrode	DPV	Fenitrothion/paddy grains and potato	0.1–7 nM	0.60 nM	[145]
PGE	Electrochemical biosensor using WO_3/g-C_3N_4 nanocomposite modified Pencil graphite electrode as immobilizing platform for *Tribolium castaneum* (Red flour beetle) acetylcholinesterase (Tc-AChE)	Amperometry	Phosmet/wheat flour	5-125 nM	3.6 nM	[146]
SPCEs	Electrochemical sensor based on strontium hexaferrite (nanorods) decorated on porous graphitic carbon nitride ($SrFe_{12}O_{19}$/g-C_3N_4) to modify SPCEs	DPV	Fenitrothion/grapes, apricots, orange, cranberry, guava, mango	0.005–378.15μM	1.4 nM	[147]
Ag-citrate/GQDs nano-ink/leaf or skin	Electrochemical sensor prepared by direct writing on the surface of the samples, using Ag-citrate/graphene quantum dots (GQDs) nano-ink.	DPV, SWV	Trifluralin/apple skin	0.005–0.04 mM	0.005 mM	[150]
PGE	Electrochemical biosensor platform developed for detection of the pesticide-DNA interaction by using disposable pencil graphite electrodes (PGEs) where DNA was immobilised via passive absorption.	DPV	Monitoring glyphosate and 2,4-dichlorophenoxyacetic acid DNA interactions	-	-	[152]
CPE	Electrochemical sensor using a carbon paste electrode modified with recrystallized zeolite	SWV	Thiram and carbendazim/honey and grape juice	0.36- $4.99x10^{-7}$M Thiram 0.10-$2.35x10^{-6}$M Carbendazim	$6.74x10^{-9}$M Thiram $3.51x10^{-9}$M Carbendazim	[154]

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NPG-GCe	Electrochemical sensor based on modified GCE with nanoporous gold film	DPV	Carbendazim and methyl parathion/water	0.5-150 µM Methyl parathion 3.0-120 µM Carbendazim	0.02 µM methyl parathion 0.24 µM Carbendazim	[155]
GCE	Electrochemical sensor based on gadolinium oxide nanorods embedded on the graphene aerogel (GdO NRs/GA)	CV	Carbendazim/water	0.01-75 µM	3.0nM	[156]
SPCEs	Electrochemical sensor based on SPCEs modified with carbon spherical shells (CSS) or Printex carbon nanoballs (PCNB)	DPV	Carbendazim and diuron/ cabbages, apples, and orange juice	0.1-1.0 µM carbendazim 1-10 µM diuron	4.7x 10^{-8} M carbendazim 9.2x10^{-7} M diuron	[157]
SPCEs	Electrochemical sensor based on SPCEs modified with carbon spherical shells (CSS) or Printex carbon nanoballs (PCNB)	SWV	Paraquat and fenitrothion /cabbages, apples, and orange juice	0.1-1.0 µM paraquat 1-10 µM fenitrothion	2.4x10^{-8}M paraquat 6.4x10^{-7}M fenitrothion	[157]

Mukherjee [136] presented a general overview of the recent advancements concerning different acetylcholinesterase (AChE) inhibition-based sensing strategies including optical, electrochemical, lab-on-paper sensors, microfluidic, and other devices for the rapid detection of organophosphorus (OPs) pesticides, developed in the last two years.

Kumar [137] focused his recent review on the biosensors developed in the last thirty years for the detection of a particular organophosphate insecticide: dichlorvos, widely used in agriculture and industry. His review described the progressive development of biosensors from the use of conventional immobilizing supports to more advanced hybrid/composite nanomaterials, also summarizing the development of biosensors by enzyme inhibition methods.

A glassy carbon electrode (GCE) modified with single-walled carbon nanohorns (SWCNH) and zein (ZE), a prolamin type-protein find in maize, was proposed by Janegitz [138] for the fenitrothion (FT) determination by means of differential pulse adsorptive cathodic stripping voltammetry (DPACSV).

Fenitrothion (FT) is an organophosphorus pesticide with cholinesterase inhibitory action, and it is widely used for insect control in grains and in vegetables cultures.

The sensor showed a linear response ranging of 9.9x10^{-7} to 1.2×10^{-5} mol L^{-1}, with a limit of detection of 1.2 ×10^{-8} mol L^{-1}. The proposed sensor was successfully applied for the determination of FT pesticide in spiked natural water and orange juice samples. Moreover, the electrochemical sensor showed good repeatability and reproducibility.

A cellulose microfibers supported reduced graphene oxide composite was employed for modifying a screen-printed carbon electrode (SPCE) for determining fenitrothion (FT) in water samples by means differential pulse voltammetry (DPV) [139].

A linear concentration range up to 1.134 mM with a detection limit of 8 nM were obtained. To validate the sensor, it was applied for the detection of fenitrothion in different spiked water samples, obtaining acceptable recovery results.

A biosensor for determining methyl parathion (MP, organophosphorus pesticide) using glutaraldehyde (Glu) cross-linked with acetylcholinesterase (AChE) immobilized on single wall carbon nanotubes (SWCNTs) enveloped with bovine serum albumin (BSA) was realized by Sundramoorthy [140].

The proposed biosensor exhibited a linear range from 1 x 10^{-10} M to 5 x 10^{-6}M with a limit of detection of 3.75 x 10^{-11} M and showed good repeatability and reproducibility. In addition, it was applied to real samples such as spiked strawberry and apple juices, obtaining good results in terms of recovery.

Poly 3,4-ethylenedioxythiophene (PEDOT) membrane and zirconia nanoparticles (ZrO_2 NPs) were directly synthesized on ITO electrode and successively employed for methyl parathion (MP) electrochemical detection [141]. Combining the individual

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properties of PEDOT (conductivity and electrocatalysis) and of ZrO_2 NPs (affinity to MP), the resulting sensor showed a limit of detection of 2.8 ng·ml^{-1} and a concentration linear range of 5–2000 ng·ml^{-1}. Furthermore, this sensor exhibited acceptable selectivity and reproducibility. The sensor was applied to spiked water sample with acceptable recovery results.

Using ultrathin MXene nanosheets (i.e., two-dimensional (2D) transition metal carbides and nitrides) as natural reducing agent and support, the shape-controlled Au-Pd bi-metallic nanoparticles via a self-reduction process, were synthesized for enhancing the performance of the resulted biosensor and to support the acetylcholinesterase immobilization. Using this multidimensional nanocomposite (MXene/Au-Pd) as functional platform, a disposable electrochemical biosensor for the detection of paraoxon, an organophosphorus pesticide was developed [142], as illustrated in figure 5.

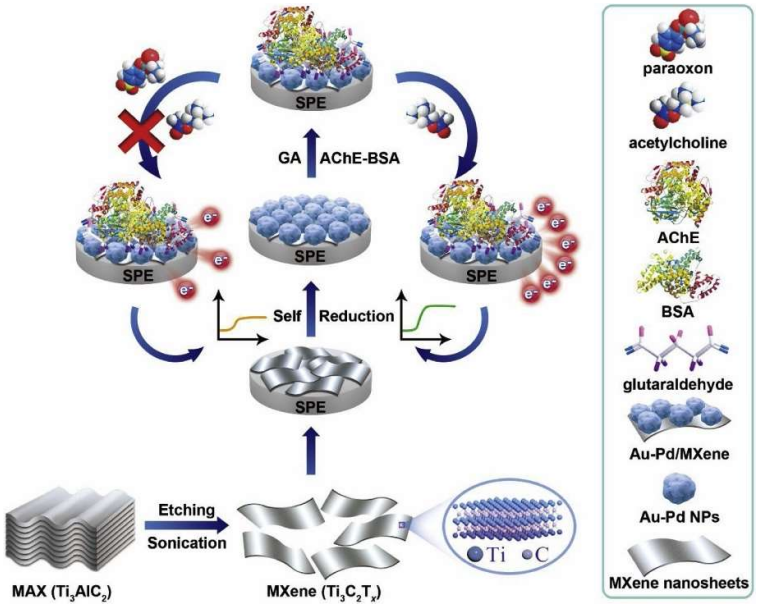

Figure 5. Schematic diagram of the synthesis of MXene nanosheets and assembling of the enzyme-based pesticide biosensor. Reprinted with permission from [142] Copyright 2020 Elsevier.

Under the optimized conditions, this biosensor showed a linear concentration range from 0.1 to 1000 µg L^{-1}, with detection limit of 1.75 ng L^{-1}. Furthermore, the biosensor was applied for paraoxon detection in spiked pear and cucumber samples with promising results.

A smart bioelectrode based on redox active protein hemoglobin (Hb) has been prepared for the determination of methylparathion (MP) [143]. The bioelectrode has been designed by immobilizing Hb on electrochemically reduced graphene oxide-chitosan based biocompatible coatings. The sensor showed a detection limit of 79.77 nM with good reproducibility.

The biosensor also was applied to spiked vegetable samples with interesting recovery results, ranging from 94% to 101%.

Another nanocomposite was synthesized via reducing graphene oxide on fumed silica (FS) surface to develop a modified electrode for the determination of fenitrothion (FT). Reduced graphene oxide (RGO) decorated with FS (FS@RGO) was used to modify glassy carbon electrode (GCE) [144].

FS on nanocomposite allowed a homogeneous distribution of the nanocomposite. Moreover, the presence of FS brings additional functionality to FS@RGO nanocomposite, which increases the adsorption and electron transfer rate of FNT. The FS@RGO/GC

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electrode showed a linear concentrations range from 0.005 to 1.0 μM and a limit of detection of 0.00019 μM. The performance of the FS@RGO/GC electrode was evaluated by means of recovery studies in river water, urine and in different fruit and vegetables extracts, (raisin, tomato, and orange) and acceptable recovery values between 92.3% and 112.2% were obtained.

An electrochemical sensor, using a glassy carbon electrode modified with a dodecane film where silver nanoparticles have been electrodeposited, was developed for fenitrothion detection [145]. The electrode was found to be stable with constant sensitivity for many cycles of analysis. The results observed in the electrochemical approach was comparable with those obtained by HPLC technique. Finally, it was applied to spiked vegetables samples such as potatoes and paddy grains.

Tribolium castaneum (Red flour beetle) acetylcholinesterase (Tc-AChE) based electrochemical biosensor integrating WO_3/g-C_3N_4 nanocomposite modified Pencil graphite electrode was developed to detect an organophosphate insecticide, Phosmet [146].

Graphitic carbon nitride g-C_3N_4 alone does not possess good electrical conductance, which can be improved by doping or coupling with other nanomaterials such as tungsten trioxide (WO_3).

WO_3/g-C_3N_4 nanocomposite provides a nontoxic, biocompatible surface for immobilizing the enzyme, providing a large surface area, high conductivity, and low ohmic resistance. The proposed biosensor showed a good analytical performance with low detection limit of 3.6 nM for Phosmet. The biosensor was also applied to detect Phosmet in spiked wheat samples with a 99% recovery rate.

In the next example, graphitic carbon nitride g-C_3N_4 was modified to improve its electrical conductivity by coupling with another nanomaterial such as strontium hexaferrite ($SrFe_{12}O_{19}$) nano-rods. The resulting nanocomposite has been used to modify screen printed carbon electrodes (SPCEs) for the detection of fenitrothion [147]. The resulted electrochemical sensor performed a good detection range from 0.005 to 378.15 μM with a low detection limit of 0.0014 μM. The $SrFe_{12}O_{19}$ /g-C_3N_4 modified sensor was applied for determination of FTN to different spiked fruits samples with acceptable recoveries.

A recent review [148] provided an overview concerning the development, applicability, and performances of nanomaterials-based immunosensors for the pesticides and herbicides detection in water, food, and soil samples.

The use of nanomaterials for the immunosensing system assembling was found to be a smart option to implement an effective and selective sensing platform for pesticides/herbicides analysis, combining different nanomaterials such as graphene, carbon nanotubes, metal nanoparticles, etc. with different sensing methodologies (e.g., electrochemical, optical, and quartz crystal microbalance (QCM)).

A recent review [149] about the determination of paraquat (PQ) in foods, including milk, apple, tomato juices, and potato samples, using electrochemical methods combined with several modified electrodes was reported by Mhammedi. Paraquat is widely used as an herbicide (broadleaf weed killer), owing to its excellent effect for crop protection and horticultural use, but it resulted very toxic and its detection, possibly on-site, is required.

The importance of the electrode modifiers combined with the most suitable electrochemical sensing technique has been underlined.

A very particular and ecofriendly method [150] has been developed for determination of trifluralin. Trifluralin is an herbicide, affecting endocrine function and so it is listed as an endocrine disruptor in the European Union list [151].

The trifluralin sensor is based on its electrochemical oxidation on a three-electrode system designed directly on the surface of an agricultural product, using Ag-citrate/GQDs (graphene quantum dots) nano-ink. The sensor was prepared by writing directly on the surface of the samples.

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Under optimized experimental conditions, this sensor was exhibited good sensitivity and specificity for trifluralin detection. The obtained linear range was between 0.008 to 1 mM and the limit of quantification was 0.008 mM, using cyclic voltammetry. Also, the obtained linear range using differential pulse voltammetry (DPV) and square wave voltammetry (SWV) is 0.005-0.04 mM with the limit of quantification of 0.005 mM. For further validation of the applicability of the proposed method, it was also used for detection of trifluralin on the surface of apple skin, as illustrated in figure 6.

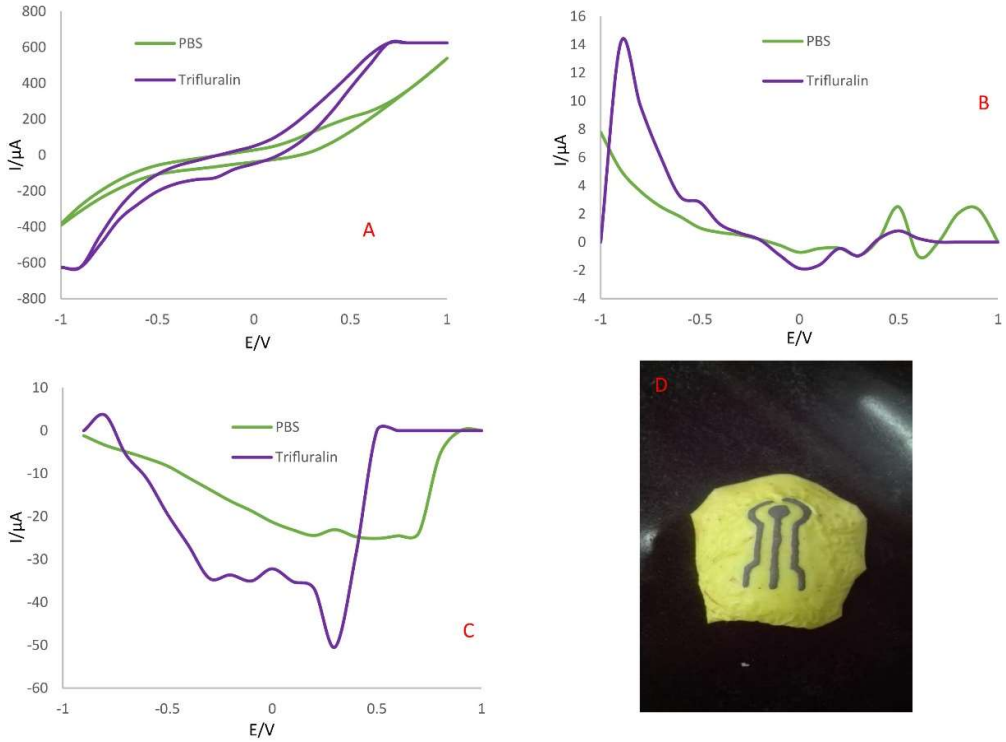

Figure 6. A) CVs, B) DPVs and C) SWVs of Ag-citrate/GQDs nano-ink fabricated on the surface of apple-skin incubated at room temperature in the absence and presence of 1 mM trifluralin. Supporting electrolyte is 0.1M PBS (pH ¼ 7.4) in the presence of acetone, D) Photographic image of electrochemical sensor made by direct writing of nano-ink on the surface of apple-skin. Reprinted with permission from [150] Copyright 2020 Elsevier.

A study concerning miniaturized lab-on-a chip platforms for on-line analysis of the pesticide-nucleic acid interactions has reported by Congur [152].

Glyphosate (GLY) is a broad-spectrum herbicide used worldwide to control grass weeds. Although it was evaluated as non-toxic agent in 20th century, its carcinogenic and genotoxic effects have been intensively investigated in the last decade. Moreover, the combination of GLY and 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely applied as herbicide mixture. Although genotoxicity of GLY has been evaluated in vivo studies, there is no report in the literature for the in vitro biointeraction monitoring of GLY and double stranded DNA, or how the combination of GLY and 2,4-D affects DNA. For this reason, an electrochemical biosensor platform was developed for investigating the pesticide-DNA interaction by using disposable pencil graphite electrodes (PGEs). First, a voltammetric investigation of the interaction between GLY and DNA was carried out. In addition, the combined genotoxic effects of the mixture of GLY and 2,4-D or the mixture of their herbicide forms onto DNA have been monitored. This effect was concentration dependent. In addition, it was evidenced that GLY as herbicide or the mixture of herbicides of GLY and 2,4-D had more genotoxic effect than analytical grade of, GLY and

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2,4-D. Finally, these disposable PGEs provide robust, eco-friendly sensing platform for monitoring of herbicide-DNA interaction with the sensitive and reliable results.

A general and recent review summarized [153] the current analytical approaches and techniques used for the analysis of Dithiocarbamate fungicides (DTFs) widely used to control fungal diffusion in crops and ornamental plants.

It included chromatography, spectroscopy, and sensor-based approaches and discussed the challenges related to selectivity, sensitivity, and sample preparation. Finally, biosensors based on enzymatic inhibition are considered very promising as analytical tools for DTFs.

A simple, inexpensive, sensitive, selective electrochemical approach was developed for simultaneous quantification of the fungicides thiram and carbendazim[154] in samples of honey, fresh grape juice, and in agricultural formulation using a carbon paste electrode modified with zeolite.

For thiram a linear concentration range of 0.36-4.99 x 10^{-7} mol L^{-1}, with limit of detection of 6.74 x 10^{-9} mol L^{-1}. For carbendazim, a linear concentration range of 0.10-2.35 x 10^{-6} mol L^{-1}, with a limit of detection of 3.51 x10^{-9} mol L^{-1}.

Thiram and carbendazim recovery experiments were performed on spiked honey and grape juice samples, yielding recovery rates in the range of 98.85-101.15%. In the agricultural formulation, the concentrations measured with the new method were close to those specified on the label, with deviations of below 1.1%. No thiram or carbendazim were found in the grape juice and honey samples. The results demonstrated the sensor applicability for quantifying both compounds simultaneously in real samples.

Nanoporous gold (NPG) with unique structural and functional properties was selected as a recognition key element of an electrochemical sensor for the simultaneous detection of methyl parathion (MP) and carbendazim (CBM) [155].

For the detection of MP and CBM, good linear responses were observed in a concentration ranges of 0.5-150 μM for MP and 3.0-120 μM for CBM, with low detection limits of 0.02 μM for MP and 0.24 μM for CBM. Additionally, the NPG/GCE electrode presented good specificity, selectivity, and it was applied for detecting the two fungicides in water samples, with interesting results in terms of recovery.

Gadolinium oxide nanorods embedded on the graphene aerogel (GdO NRs/GA) were employed for assembling a selective electrochemical sensor to detect carbendazim (CBM) [156].

The GdO NRs/GA-modified electrode showed good analytical performances. Interestingly, the GdO NRs are strongly anchored in the GA matrix, providing an efficient pathway for the rapid electron transfer. A linear concentration range from 0.01 to 75 μM with a low detection limit of 3.0 nM has been achieved The sensor was applied to spiked water sample and the results are comparable with those obtained from HPLC technique, with a recovery ranging from 97.80–99.40%.

An interesting example for pesticides on-site monitoring in food is reported by Raymundo-Pereira [157]. It is a non-enzymatic electrochemical sensor, but the approach seems to be particularly innovative and, on my opinion, worthy to be mentioned and highlighted.

A set of three glove embedded sensors printed on three fingers of a rubber glove allowed the selective, sensitive, and simultaneous detection of different pesticides such as carbendazim (carbamate),diuron (phenylamide), paraquat (bipyridinium) and fenitrothion (organophosphate). In figure 6 are illustrated the design and working principle of the glove-embedded sensors.

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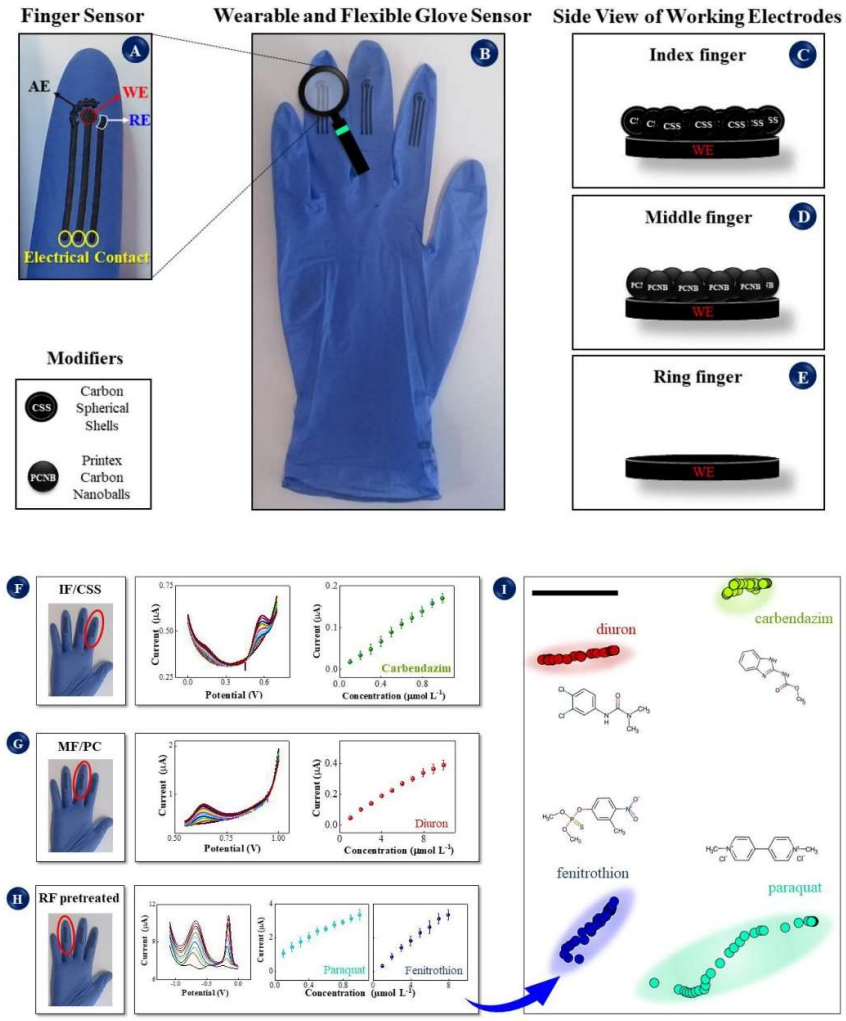

Figure 7. Details of the design and working principle of the glove-embedded sensors to detect the pesticides. Reprinted with permission from [157] Copyright 2021 Elsevier.

The sensors consisted of a pre-treated screen-printed carbon electrode and two other such electrodes coated with either carbon spherical shells (CSS) or Printex carbon nanoballs (PCNB). Detection of carbendazim and diuron was performed using differential pulse voltammetry (DPV) and the electrodes coated with CSS and PCNB, respectively, with limits of detection of 4.7×10^{-8} and 9.2×10^{-7} mol L^{-1}. Square wave voltammetry (SWV) was applied to detect paraquat and fenitrothion with limits of detection 2.4×10^{-8} and 6.4×10^{-7} mol L^{-1} using the pretreated electrode in sulfuric acid solution. To investigate the applicability of the sensors to food real samples, spiked samples of cabbages and apples, and orange juice were analyzed. The finger contacted the food sample during the analysis.

The recoveries varied between 90 and 110% indicating that the glove-based sensors are selective and effective for the detection of carbendazim, diuron, paraquat and fenitrothion in real food samples. The interference from other pesticides such as chlorpyrifos, carbaryl, metomyl, atrazine, trifluralin, glyphosate and chloranil was investigated and resulted negligible from the experimental evidence.

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3.4 Antibiotics

Antibiotics are a group of pharmaceutical drugs widely used in human and veterinary medicine for treating different many infectious diseases [158].

Large quantities of antibiotics are used annually in livestock industry and aquaculture worldwide, and antibiotic use for animals can produce antibiotic residues in food products such as meat, chicken, egg, milk, honey, and fish [159].

Residues of these drugs can induce several toxic effects in humans [160]. The most common side effect of antibiotics residues in foodstuffs is the development of antimicrobial resistance. The resistant bacterial pathogens can be transferred to human through the food chain and cause the inefficiency of antibiotic therapy [159, 160].

In order to minimize the adverse effects of antibiotics, the European Union has banned some specific antimicrobial, while for those not banned, Maximum Residue Limits (MRLs) have been established to ensure consumers safety from ingestion of antibiotic residue in animal-derived foods [161].

To guarantee that the residue of antibiotics in animal-derived foods is below the MRL, it is very important to find suitable methods to determine the content of antibiotics.

Several reviews reported the biosensing approaches for antibiotics detection. In particular, Marty presented [162] and highlighted the achievements in the development of biosensors in the above-mentioned application field, evidencing the different types of involved nanomaterials and the biorecognition elements .

Very recently Liang [163] revised the currently antibiotic detection technologies including chromatography, mass spectrometry, capillary electrophoresis, optical detection, and electrochemistry and evidencing the advantages and drawbacks.

Herein, we reported the most recent advances in electrochemical biosensors for the detection of antibiotics in food and table 4 summarizes the analytical characteristics of the electrochemical biosensors for antibiotics reported in the review.

Table 4. An overview of recent electrochemical biosensors for antibiotics determination.

Electrode	(Bio)sensor format	Electrochemical technique	Sample/analyte	L-R.	LOD	References
GCE	Multiplexed electrochemical aptasensor using metal ions encoded apoferrtin probes and double stirring bars-assisted target recycling for signal amplification	SWV	Kanamycin and ampicillin/milk and fish	0.05pM-50 nM	Kanamicin 18fM Ampicillin 15fM	[[164]
AuE	Electrochemical aptasensor based on the application of a ladder-shaped DNA structure as a multi-layer physical block on the surface of gold electrode	DPV	Ampicillin/milk	7pM-100 nM	1 pM	[165]
Thin film gold electrode (TFGE),	Disposable and portable aptasensor using gold nanoparticles (AuNPs)/carboxylated multi-walled carbon nanotubes (cMWCNTs)@thionine connecting complementary strand of aptamer (cDNA) as signal tags	DPV	Oxytetracycline/ chicken	1×10^{-13}-1×10^{-5} gmL^{-1}	3.1×10^{-14} gmL^{-1}	[166]
GCE	Electrochemical aptasensor based on protective effect of aptamer-antibiotic conjugate towards endonuclease DpnII activity.	DPV	Ampicillin/milk and water	0.1–100 nM	32 pM	[167]
AuE	Electrochemical aptasensor incorporating elements of triple-helix aptamer probes (TAP), catalyzed hairpin assembly (CHA) signal amplification and host-guest recognition	DPV	Tetracycline/milk	0.2-100 nM	0.13 nM	[168]
SPAuE	Electrochemical aptasensor based on aptamer cocktail on the surface of gold screen-printed electrodes	DPV	Tetracycline/honey	0.01–1000 $ngmL^{-1}$	0.0073 $ngmL^{-1}$	[169]

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AuE	Electrochemical aptasensor based on the classical probe conformation changing mode (PCCM) with a methylene blue (MB) label used as an electrochemical tag	SWV	Kanamicin/milk and water	10.0 nM-10.0 μM	3 nM	[170]
AuE	Electrochemical aptasensor based on the target-induced signal probe shifting (TISPS) method with a free MB label in the assay solution.	SWV	Kanamicin/milk and water	200.0 pM-1.0 μM	60 pM	[170]
SPCEs	Potentiometric aptasensor array based on a 4-channel screen-printed carbon electrode	OCP (Open Circuit Potential) measurement	Streptomycin and kanamycin/milk	Streptomicin 10 pM-10 μM Kanamicin10 pM-1 μM	Streptomycin 9.66 pM Kanamycin 5.24 pM	[171]

A multiplexed electrochemical aptasensor for multiple antibiotics detection, using kanamycin (KANA) and ampicillin (AMP) as model analytes, was assembled by using metal ions encoded apoferritin probes and double stirring bars-assisted target recycling for signal amplification [164].

KANA and AMP have been determined simultaneously within the range from 0.05 pM to 50 nM and the detection limits were 18 fM KANA and 15 fM AMP. The feasibility of the aptasensor was evaluated by testing milk and fish samples. Spiked samples with KANA and AMP were employed and the analytical results were coherent and comparable with those obtained by ELISA.

The sensing approach of an electrochemical aptasensor [165] for detection of ampicillin (AMP) is based on the application of a ladder-shaped DNA structure as a multi-layer physical block on the surface of gold electrode. A sensitive detection of AMP was obtained with a detection limit of 1 pM, probably due to the electrostatic repulsion and physical hindrance of the ladder-shaped DNA structure.

The aptasensor response was linear in the concentrations range from 7 pM to 100 nM. The aptasensor was applied to spiked milk samples with satisfactory results.

He [166] developed a disposable and portable aptasensor for the fast and sensitive detection of oxytetracycline using gold nanoparticles (AuNPs)/carboxylated multi-walled carbon nanotubes (cMWCNTs)@thionine connecting complementary strand of aptamer (cDNA) as signal tags. The substrate electrode of the aptasensor was a portable thin film gold electrode (TFGE).

In the presence of OTC, OTC competed with cDNA to combine with aptamer. The bioconjugate (AuNPs/cMWCNTs/cDNA@thionine) was released from the TFGE and the electrochemical signal decreased.

Under optimized conditions, the aptasensor showed a dynamic range of 1×10^{-13}–1×10^{-5}gmL^{-1} for OTC with a low detection limit of 3.1×10^{-14} gmL^{-1} and was applied for the determination of OTC to spiked chicken samples with satisfactory performances.

Another aptasensor, based on the protective effect of aptamer-antibiotic conjugate towards endonuclease DpnII activity, was developed [167] for determining ampicillin in milk and water samples.

Ampicillin is detected with low detection limit of 32 pM under optimal conditions, which is lower than the MRL allowed by the European Union (9.93 nM, 4 μgkg^{-1}).). The developed method also presents good selectivity. Moreover, the applicability is tested by detecting antibiotic in spiked milk and water samples with satisfactory results.

A novel "signal-on" sensing strategy for sensitive electrochemical determination of tetracycline (TC) was reported [168] for the first time, including elements of triple-helix aptamer probes (TAP), catalyzed hairpin assembly (CHA) signal amplification and host-guest recognition. Under optimal conditions, a linear relation along with the logarithm of the TC concentrations ranging from 0.2 nM to 100 nM, and ta detection limit of 0.13 nM.

The sensor was employed in spiked milk samples to evaluate the recovery. It ranged from 92.8% to 107.7% and can be considered satisfactory.

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An aptamer cocktail was immobilized on the surface of gold screen-printed electrodes for developing an electrochemical aptasensor for detection of tetracycline (TC) in honey [169], as shown in figure 8. The aptamer cocktail was composed by a comparatively short aptamer (Apt40) and a comparatively long aptamer (Apt76), with a different base composition, different chain lengths, and different folded binding sites.

The aptasensor provided a detection limit of 0.0073 ng mL^{-1} and a linear concentration range from 0.01 to 1000 ng mL^{-1}. When detecting TC in spiked honey samples, the aptasensor showed high specificity and good recovery rates of 96.45-114.6%. This aptasensor can represent a model for developing aptasensors towards other targets.

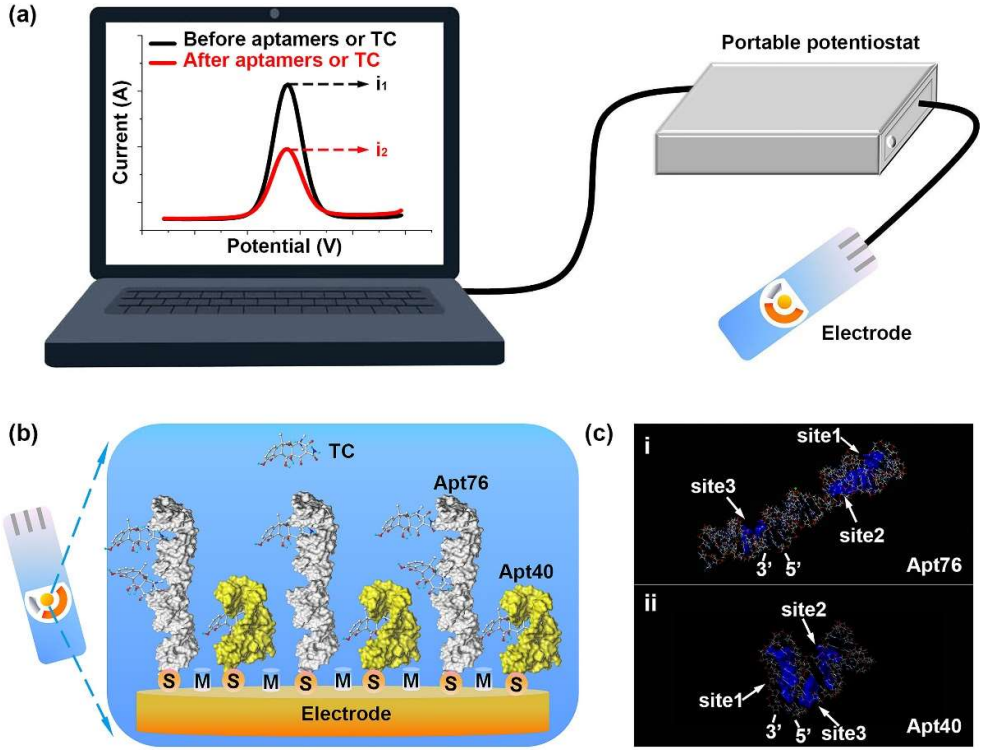

Figure 8. Schematic illustration of the aptamer cocktail-based electrochemical aptasensor. Reprinted with permission from [169] Copyright 2019 Elsevier.

Two typical kanamycin (KAN) electrochemical aptasensors employing different signal transduction mechanisms were designed and assembled with a similar structure [170]. One sensor (sensor-1)was based on the so-called classical probe conformation changing mode (PCCM) with a methylene blue (MB) label used as an electrochemical tag. The other sensor (sensor-2) used the target-induced signal probe shifting (TISPS) method with a free MB label in the solution. The difference in signal transduction mechanisms resulted in differences in the electrochemical behavior and sensing performance. The results show that both sensor types exhibit different electrochemical behavior in square wave voltammetry, cyclic voltammetry, and in sensitivity, with detection limits of 3.0 nM for sensor-1 and 60.0 pM for sensor-2 in buffer. When validated for detection of tap water and milk samples, both sensing methods showed good performances with detection limits of <260 nM and measurement times of <40 min. In addition, accuracy was good with mean recoveries of 72.3–92.6%.

Compared with PCCM, TISPS is more conducive with a low background signals, improving the sensitivity, but has a little bit slower response and weaker anti-fouling ability in complex matrix. Both sensors present their respective advantages justifying their development for the detection of KAN.

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A novel potentiometric aptasensor array based on a 4-channel screen-printed carbon electrode was developed with a dual-internal calibration system for the simultaneous detection of streptomycin and kanamycin [171]. Two channels were used as working channels for assembling the aptamers of the two targets, and the other two channels were calibration channels.

Under optimal conditions, this aptasensor array showed high sensitivity for the detection of streptomycin and kanamycin with detection limits of 9.66 pM and 5.24 pM, respectively, and corresponding linear response ranges of 10 pM–10 μM and 10 pM–1 μM, respectively.

Moreover, it presented good specificity without interference between the two targets or with other antibiotics and also exhibited good repeatability. This aptasensor array was further applied to the simultaneous detection of streptomycin and kanamycin in real milk samples, and the results were validated by liquid chromatography-mass spectrometry (LC–MS).

3.5 Endocrine-Disrupting Chemicals

Endocrine-disrupting chemicals (EDCs) are environmental contaminants/pollutants and are also known as hormone disrupting compounds [172], WHO is particularly sensitive to the problem of the presence and determination of endocrine disrupters [173].

Furthermore, EDCs represent a broad class of molecules such as pesticides (see for example, trifluralin [151]) and industrial chemicals, plastics and plasticizers, fuels, and many other chemicals, present in the environment.

Herein, we focused our attention on Bisphenol A (BPA) and on the estrogens and table 5 summarizes the analytical characteristics of the electrochemical biosensors for BPA and estrogens reported in the review.

Table 5. An overview of recent electrochemical biosensors for BPA and estrogens determination

Electrode	(Bio)sensor format	Electrochemical technique	Sample/Analyte	L-R.	LOD	References
Interdigitated electrode (IDE)	Label free impedimetric aptasensor printed circuit board (PCB) technique	EIS	BPA/canned food	1 fM-10 pM	152.93 aM	[175]
BDDE	Impedimetric aptasensor based on Au nanoparticles (Au-NPs) coated boron-doped diamond (BDD) modified with aptamers, and 6-mercapto-1-hexanol (MCH)	EIS	BPA/milk	$1x10^{-14}$-$1x10^{-9}$M	$7.2x10^{-15}$M	[176]
AuE	Label-free electrochemical aptasensor based on functionalized multiwall carbon nanotubes/gold nanoparticles(f-MWCNTs/AuNPs) nanocomposite film modified gold electrode	SWV	BPA/mineral water, orange juice, milk	0.1–10 nM	0.05 nM	[177]
GCE	Electrochemical sensor using hierarchical Ce-metal-organic framework (Ce-MOF) modified with cetyltrimethylammonium bromide (CTAB) as a sensing platform.	DPV	BPA/milk	0.005-50μM	2 nM	[178]
GCE	Electrochemical sensor based on the AuPd nanoparticles incorporated	DPV	BPA/milk	0.18–18 μM	60 nM	[179]

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	carboxylic multi-walled carbon nanotubes (MWCNT)					
GCE	Electrochemical aptasensor based on MWCNT/SiO2@Au nanocomposite.	SWV	BPA/water, orange juice, milk	0.1-100μM	10 pM.	[180]
GCE	Electrochemical sensor using as sensing platform multi walled carbon nanotubes and chitosan (MWCNTs-CH) self-assembled on graphene nanoplatelets GNPs (GNPs-MWCNTs-CHIS)	DPV	BPA/milk	0.1–100 μM	0.05	[181]
GCE	Electrochemical sensor based on three-dimensional hierarchical cylinder-like nickel nanoparticle/nitrogen-doped carbon nanosheet/chitosan nanocomposite (NiNP/NCN/CHI)	DPV	BPA/milk	0.1-2.5 μM and 2.5-15.0 μM	45 nM	[182]
BDDE	Electrochemical sensor, using a cathodically-pretreated boron-doped diamond (Cpt-BDD)electrode in combination with QuEChERS extraction method	SWV	E,E-dienestrol/fish tissue	$2.30x10^{-7}$-9.69×10^{-6} M	$5.43x10^{-8}$ M	[188]
SPCE	Impedimetric Aptasensor Based on Carbon Nanodots Modified SPC Electrode	EIS	17β-Estradiol/water	$1.0x10^{-7}$-$1.0x10^{-12}$ M,	$0.5x10^{-12}$ M.	[189]
AuE	Electrochemical biosensor based on graphene quantum dots (GQDs)/conducting polymer and laccase modified gold electrodes	CV	17β-Estradiol/no real samples	$0.1\text{-}120x10^{-6}$ M	1μM	[190]

Bisphenol A (BPA) is a synthetic chemical, classified as a non-biodegradable compound with high chemical resistance, and widely used as monomer in the synthesis of epoxy resins and polycarbonate.

Due to their properties polycarbonates have different application such as in fabrication of water bottles, infant feeding bottles, toys, utensils, thermal paper, and medical equipment . For similar reasons, epoxy resins are widely used as protective coatings for food and beverage containers, paints, adhesives, and electronic laminates. In both cases, BPA can contaminate food commodities and water.

Being an endocrine disruptor, BPA can cause serious adverse effects on human health even at very low concentrations [172, 173]. BPA has a similar structure to that of estradiol and diethylstilbestrol and thus can stimulate cellular response, binding with the estrogen receptors.

Because of its serious adverse effects, it is required to develop a reliable, remarkable selective and sensitive analytical method for BPA identification.

Recently many efforts have been made to develop rapid, simple, sensitive, and field-portable alternatives for BPA detection, considering that the conventional methods require complex pretreatments of the sample, long time for the analysis and skilled personnel.

Verdian presented [174] a comprehensive overview on recent developed aptamer-based biosensors for the detection of BPA. In addition, trends in the development of colorimetric, fluorescence and electrochemical aptasensors for the monitoring of BPA are shown so that they can give a new idea for designing commercial kits.

A label-free impedimetric aptasensor for the detection of BPA was developed using a BPA specific aptamer as probe molecule [175]. The developed biosensor can detect BPA

level in 20 s and exhibits a linear range from 1 fM to 10 pM, with a limit of detection of 152.93 aM. This biosensor was applied to test BPA in spiked canned food samples with good recovery results.

Li [176] developed an electrochemical impedance aptasensor based on Au nanoparticles (Au-NPs) coated boron-doped diamond (BDD) electrode modified with aptamers, and 6-mercapto-1-hexanol (MCH) for the detection of BPA. It showed good linearity from $1.0x10^{-14}$ to $1.0x10^{-9}$ mol L^{-1}. The detection limit of 7.2×10^{-15} mol L^{-1} was achieved, which can be attributed to the synergistic effect of combining BDD with Au-NPs, aptamers, and MCH. The results of BPA analysis in buffer and in milk indicated good sensitivity, specificity, stability, and repeatability of the aptasensor.

Another label-free electrochemical aptasensor was realized [177] for determination of bisphenol A, based on functionalized multiwall carbon nanotubes/gold nanoparticles (f-MWCNTs/AuNPs) nanocomposite film modified gold electrode. Under the optimized experimental conditions, a linear concentrations range from 0.1to 10 nM with a detection limit of 0.05 nM. The effect of interfering species was investigated and the proposed aptasensor resulted selective for BPA. In addition, the reproducibility and stability of the sensor were satisfactory. Finally, the developed aptasensor was successfully applied to spiked real samples such as mineral water, orange juice, and milk, with results acceptable in terms of recovery

Zhang [178] prepared an electrochemical bisphenol A sensor using hierarchical Ce-metal-organic framework (Ce-MOF) modified with cetyltrimethylammonium bromide (CTAB) as a sensing platform. Ce-MOF was synthesized and modified with a cationic surfactant (CTAB) via an electrostatic interaction. CTAB/Ce-MOF suspension was used to modify a glassy carbon electrode (GCE), so obtaining as final sensor a CTAB/Ce-MOF/GCE. A linear concentrations range from 0.005 to 50 μmol L^{-1} and a low detection limit of 2.0 nmol L^{-1}. The proposed sensor showed a good reproducibility, stability, and anti-interference behavior, and was applied for BPA detection to spiked milk samples, with acceptable recovery results ranging from 96.2 to 104.6%.

An interesting electrochemical sensor for BPA based on the AuPd nanoparticles incorporated in carboxylic multi-walled carbon nanotubes (MWCNT) was designed and assembled by Liu [179], where MWCNT improved electron transfer and poly (diallyldimethylammonium chloride) (PDDA) acted as dispersing agent for MWCNT and for further increasing the metal NPs loading. A linear concentrations range from 0.18–18 μM and the detection limit of 60 nM were determined. The sensor showed good sensitivity, stability, repeatability and can be used to detect BPA in spiked milk and water samples with good performance in terms of recovery.

A sensitive electrochemical aptasensor was developed [180] for the detection of BPA, based on MWCNT/SiO_2@Au nanocomposite. The detection strategy is based on $[Fe(CN)_6]^{3-/4-}$ as a label free redox probe. In the absence of BPA, the aptamers remain unfold. After the BPA addition, strong interactions between the analyte and the aptamer are evidenced and an electrochemical signal decrease is occurred. The proposed electrochemical aptasensor was selective with a linear concentrations range from 0.1 to 100 nM and a limit of detection of 10 pM. This aptasensor was successfully applied for detection of BPA to spiked water, orange juice and milk samples obtaining acceptable recovery results ranging from 96 to 104%.

Graphene nanoplatelets (GNPs), multiwalled carbon nanotube (MWCNTs) and chitosan (CS) were self-assembled by a one-step hydrothermal reaction and a novel MWCNTs-CS enfolded GNPs (GNPs-MWCNTs-CS) composite was synthesized and used to modify a glassy carbon electrode (GCE).

The GNPs-MWCNTs-CS/GCE was employed as sensing platform to determine BPA by differential pulse voltammetry (DPV) [181]. Under the optimum conditions, a linear for the current concentrations range from 0.1 to 100 μM with a detection limit of 0.05 nM is observed.

The proposed sensor showed a good selectivity, repeatability, and reproducibility and it was applied to different spiked milk samples with interesting results in terms of recovery.

As last, but not least example, a three-dimensional hierarchical cylinder-like nickel nanoparticle/nitrogen-doped carbon nanosheet/ chitosan nanocomposite (NiNP/NCN/CS) was used for modifying a glassy carbon electrode (GCE) to assemble a sensing platform for BPA determination [182].

Two linear concentrations ranges were observed, the first from 0.1 to 2.5 μM and the second from 2.5 to 15.0 μM. The detection limit for BPA detection is estimated to be 45 nM. Finally, the BPA sensor showed a good selectivity, and stability, and it was employed to detect BPA in spiked milk samples, with recoveries ranging from 96 to 105%.

Among the endocrine-disrupting chemicals, environmental estrogen is a type of endocrine disruptor, able to interfere with the hormone metabolism in the human organisms, thereby affecting physiological functions such as growth, development, and reproduction. Typically, environmental estrogens are divided into naturally produced, such as 17β-estradiol (E2), estrone (E1), and estriol (E3), and synthetic forms, such as bisphenol A (BPA) [172, 173], 17α-Ethinylestradiol (EE2), diethylstilbestrol (DES), and others

Natural estrogens are synthesized in the human and animal organism, and synthetic estrogens are generally employed as active agents in contraception or hormone therapy [183]. These estrogens can penetrate in the waterways after excretion from human and animals, adding to the natural estrogens and veterinary drugs excreted by livestock in many rural areas. In this context, it is inevitable that estrogens can access in the food chain and affect public health. Due to their lipophilicity, estrogens can accumulate in the adipose tissues. [184]. The exogenous estrogens are very slowly eliminated, so interfering in the function, and metabolism of the endocrine system. Therefore, it is of great importance to monitor exogenous estrogen contamination in water and food.

Very recently, interesting reviews focused on methods concerning the determination of estrogens in food matrices have been published.

In particular, Gunatilake [185] revised the novel methodological developments for the determination of five steroidal estrogens, estriol, 17α-estradiol, 17β-estradiol, estrone, and ethinyl estradiol in food matrices including dairy products, fish, meat. In addition, significant attention has been given to methods and analytical approaches which allow to directly determine the contaminant, optimizing analysis time and protocols.

Bala [186] reported an overview concerning the recent advances in electrochemical sensors based on electrochemical impedance spectroscopy for the detection of endocrine disruptors, including synthetic estrogens. In this review, the fact that EIS -based sensors can be easily implemented in fully automated devices by integrating electrodes in microfluidic chips, has been emphasized.

Finally, Sun [187] described extensively the recent developments in biosensors for the detection of estrogens in the environment and food, including molecule-based biosensors, cell-based biosensors, and model organism-based biosensors.

In particular, works published in 2017-2019 and focused on methods for the detection of estrogens and the use of nanomaterials for biosensors development have been considered, evidencing the advantages and limitations of the different kind of biosensors.

Herein, we reported some newly released examples of electrochemical (bio)sensors for estrogen determination.

Oliveira [188] and co-workers described a promising electrochemical sensor to monitor the synthetic estrogen E,E-dienestrol (E,E-DNL) in fish tissue, using a cathodically pretreated boron-doped diamond (Cpt-BDD) electrode in combination with QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method.

A linear concentration range from 2.30×10^{-7} to 9.69×10^{-6} mol L^{-1} of E,E-DNL, with a detection limit of 5.43×10^{-8} mol L^{-1}, good repeatability and reproducibility test was evidenced. The procedure was successfully applied to quantify E,E-DNL in QuEChERS

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extracts from Nile Tilapia (*Oreochromis niloticus*) liver tissue with a recovery ranging from 92.3 to 98.8%.

Haniphah [189] designed and assembled a simple and sensitive impedimetric aptasensor based on conductive carbon nanodots (CDs) for the detection of 17β-Estradiol (E2). Carbon nanodots were electrodeposited on a screen-printed electrode (SPE), acting as platform for immobilizing 76-mer aptamer probe. The impedimetric aptasensor exhibited a linear concentration range from 1.0 x 10^{-7} to 1.0 x 10^{-12} M, with a detection limit of 0.5 x 10^{-12} M. The developed biosensor showed high selectivity toward E2 in the presence of progesterone (PRG), estriol (E3) and bisphenol A (BPA), respectively.

Moreover, the average recovery rate for spiked river water samples ranged from 98.2% to 103.8%, evidencing the aptasensor possible application for E2 detection in water samples.

Finally, two biosensor based on graphene quantum dots (GQDs)/ laccase gold (Au) electrodes was developed by Cabaj and co-workers [190]. The process of hormone determination was based on the redox reaction catalyzed by laccase enzyme.

Under optimized conditions, the biosensor showed a linear range from 0.1–120x10^{-6} M) with a detection limit of about 1 μM. Moreover, the method was successfully applied for hormone determination in the presence of interfering compounds such as ascorbic acid, L-cysteine, uric acid. As a final comment, an investigation of the biosensor applicability to real samples, e.g., in water should be important.

3.6 Allergens

Anomalous reactions due to the food ingestion are defined as "adverse reactions to food". They are classified by the European Academy of Allergology, and Clinical Immunology, based on the response mechanism, as toxic and non-toxic reactions [191]. Toxic reactions are connected with a food primary harmful effect after the ingestion. Non-toxic reactions depend on individual sensitivity, are not commonly dose dependent, and are classified as immunological (food allergy) and non-immunological (food intolerance) [192, 193]. Food allergy is an adverse immune-mediated response occurring after an ingestion/ exposure to a given food, component, or ingredient.

Food allergens represent a major food safety concern in industrialised countries. The European Union has established labelling rules for 14 allergenic food ingredients i.e., eggs, milk, peanuts, nuts, gluten-containing cereals, lupin, soybeans, celery, mustard, sesame seeds, fish, crustaceans, mollusks, and sulphites: therefore, it is mandatory to label them on their food derivatives [194] Although food labelling is required for providing consumers with composition information, accidental ingestion/exposure to some allergen can occur. This exposure can be due to undeclared allergens through adulteration, cross-contamination, or even fraud.

Considering the scenario described above, it is clear that precise, cost effective and fast analytical methods are required for a reliable screening of specific allergens in food commodities and electrochemical biosensors seem to meet all these requirements, including on-site analysis, and involving unskilled personnel.

Several reviews reported the (bio)sensing approaches for determination of foods allergens.

In particular, Marty and co-workers [195] highlighted the success of the application of electrochemical affinity biosensors based on disposable screen-printed electrodes for the detection of allergens, also reporting some interesting examples for specific allergens.

Pingarron and his group [196] presented the most significant trends and developments in electrochemical affinity biosensing in this field over the past two years as well as the challenges and future prospects for this technology.

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Conte-Junior [197] in his review underlined that the integration of biosensor and nanoparticles is very promising for the accurate and reliable analysis of allergenic proteins in the food samples.

Finally, Maquieira [198] reviewed recent approaches, including the electrochemical biosensors, existing kits for food-borne allergen detection and cutting-edge applications by focusing on the sensitivity, selectivity, and applicability of current methods in food samples.

Herein, we reported significative examples of electrochemical (bio)sensors for the detection of allergens and table 6 summarizes the analytical characteristics of the electrochemical biosensors reported in the review

Table 6. An overview of recent electrochemical biosensors for allergens determination

Electrode	(Bio)sensor format	Electrochemical technique	Analyte/ Sample	L.-R.	LOD	References
GCE	Electrochemical immunosensor based on zein polymer coupled with carbon nanotubes as sensing platform to immobilize capture antibody	SWV	Gliadin/Wheat flour	0.5-100 ppm	0.5 ppm	[199]
SPCE	Microfluidic electrochemical aptasensing system based on a combination of 2D nanomaterial molybdenum disulfide (MoS_2) and graphene with the addition of gold nanoparticles.	DPV	Gliadin/Wheat flour	4-250 nM	7 pM	[200]
GSPE	Electrochemical aptasensor based on poly(aniline-*co*-anthranilic acid) (PANI/PAA) composite polymer coupled with a specific aptamer	DPV	*β*-lactoglobulin/milk	0.01–1.0 μgmL^{-1}	0.053 μgmL^{-1}	[202]
GSPE	Electrochemical Aptasensor based on Poly-L-Lysine Modified Graphite Electrodes	DPV	*β*-lactoglobulin/milk, yogourt	0.1–10 $ngmL^{-1}$	0.09 $ngmL^{-1}$.	[203]
SPAuE	Electrochemical label free immunosensor using polypyrrole (PPY) electropolymerized as immobilizing platform for the capture antibody	DPV	*a*-lactoglobulin/meal	355–2840 $pgmL^{-1}$	0.192 $fgmL^{-1}$	[204]
ITO	Electrochemical aptasensor based on a highly selective DNA aptamer and flower-like $Au@BiVO_4$ microspheres	Amperometry	*β*-lactoglobulin/infant food formula	0.01-1000 ng mL^{-1}	0.007 $ngmL^{-1}$	[205]
GSPE	Electrochemical immunosensor based on gold-nanocluster-modified graphene screen-printed electrodes	DPV	*β*-lactoglobulin/milk	0.01-100 $ngmL^{-1}$	0.08 $ngmL^{-1}$	[206]
PGE	Electrochemical sensor based on graphene oxide modified pencil graphite electrode	CV	*β*-lactoglobulin/milk	530-11.160 μgL^{-1}	270 μgL^{-1}	[207]
SPCE	Disposable amperometric magnetoimmunosensor using a sandwich configuration involving selective capture and detector antibodies and carboxylic acid-modified magnetic beads (HOOC-MBs).	Amperometry	Ara h 2/ flour	87-10.000 pg mL^{-1}	26 pg mL^{-1}	[208]
GCE	DNA biosensor based on gold-palladium nanowaxberries (AuPd NWs)/dodecylamine functionalized graphene quantum dots(D-GQDs)-graphene micro-aerogel(GMA) composite.	DPV	Ara h 1/ peanut milk	$1.0x10^{-22}$-$1.0x10^{-17}$M	$4.7x10^{-23}$M	[209]
SPCE	Paper-based capacitance mast cell sensor based on 3D paper chip printed with carbon electrodes as a non-contact capacitance sensing platform, using a polyvinyl alcohol (PVA)-gelatin methacryloyl (GelMA)-nano-hydroxyapatite (nHAP) composite	Capacitance measurement	Ara h 2/ raw and fried peanut	0.1-100 $ngmL^{-1}$	0.028 $ngmL^{-1}$	[210]

	hydrogel (PGHAP gel) to improve the conductivity and biocompatibility of the cellulose paper,					
Magnetic glassy carbon electrode (MGCE)	Cell sensor, based on fluorescent magnetic beads	EIS	Tropomyosin and parvalbumin/Crucian carp and brown shrimp	-	Tropomyosin 0.03 μgmL^{-1} Parvalbumin 0.16 $ngmL^{-1}$	[211
SPCE	Label free electrochemical immunosensor assembled by electrochemically reducing 4-carboxyphenyl diazonium salt, which had been electrochemically generated in situ, to a stable 4-carboxyphenyl layer on carbon nanofiber-modified screen-printed electrode.	DPV	Porcine serum albumin/ pork fresh meat	0.5-500 $pgmL^{-1}$	0.5 $pgmL^{-1}$	[212]
CE	Electrochemical sensor using molecularly imprinted polymers (MIPs) for detecting genistein, an allergenic soy marker.	DPV	Genistein/no real samples	100ppb-10ppm	100 ppb	[213]

The starting examples are focused on the design and assembling of biosensors for detecting the protein gliadin, responsible for serious autoimmune disorder causing chronic diarrhea, fatigue, weight loss and anemia in celiac people.

The first example is a biosensor where a natural polymer zein, coupled with nanomaterials such as carbon nanotubes, acts as a natural platform for anchoring the capture antibody onto the glassy carbon electrode (GCE) [199]. GCE was functionalized through a layer-by-layer deposition of zein and carbon nanotubes (Z-CNT) nanocomposite, where Z-CNT behaves as a natural linker molecule with several functional groups for immobilizing capture antibody and target, guaranteeing good sensor performances.

The Z-CNT biosensor showed a detection limit of 0.5 ppm, a linear concentrations range from 5 to 100 ppm, good selectivity for gliadin, towards other food toxins and good stability. In addition, it was also applied to wheat flour samples, the content of gliadin was examined after its extraction from the flour samples and the extracts were analysed, with acceptable results in terms of accuracy and specificity.

Singh [200] proposed a microfluidic electrochemical aptasensing device for the detection of gliadin. The sensor assembling involves the combining use of a 2D nanocomposite involving molybdenum disulfide (MoS_2)/graphene and gold nanoparticles. Aptamers, specific for gliadin, were used as biomarkers. A polydimethylsiloxane (PDMS)-based flexible microfluidic device integrated the sensor. The aptasensor showed a limit of detection was 7 pM and a good linear range was observed from 4 to 250 nM. Samples of rice flour, naturally gluten free, spiked with wheat flour, were tested and a good recovery was observed between 98 and 102%.

Among the food allergies, cow milk allergy is one of the most common form of childhood allergy and unfortunately this kind of allergy can persist for life.

Marrazza and her group have recently published an interesting review and some papers concerning the detection of milk allergens by means of electrochemical biosensors.

The review [201] focused on particular research advances in biosensors (specifically immunosensors and aptasensors) for detection of the milk allergens. Different allergic proteins of cow milk are described together with the analytical standard methods for their detection. Additionally, the commercial status of biosensors is also discussed in comparison to conventional techniques like enzyme-linked immunosorbent assay (ELISA)

The same group has developed [202] an electrochemical disposable platform based on poly(aniline-co-anthranilic acid) (PANI/PAA) copolymer coupled with an aptamer for the detection of β-lactoglobulin, the main cause of the milk infant allergy. After optimization of experimental parameters, a dose-response curve was obtained between 0.01–1.0 μgmL^{-1} β-lactoglobulin concentration range with a limit of detection of 0.053 μgL^{-}

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[1]. Milk samples spiked with β-lactoglobulin were analysed with a recovery range between 80 and 95%.

Another sensing methodology always for the determination of β-lactoglobulin in food samples was designed by Marrazza group [203] using a folding-based electrochemical aptasensor based on poly-L-lysine modified graphite screen-printed electrodes (GSPEs) and an anti-β-lactoglobulin aptamer tagged with methylene blue (MB). This aptamer changes its conformation when the sample contains β-LG, and this induces changes in the distance between MB and the electrode surface and consequently in the electron-transfer rate. The response of this biosensor was linear for concentrations of β-LG within the range 0.1–10 ng mL^{-1}, with a limit of detection of 0.09 ng mL^{-1}.

The aptasensor performance were evaluated on spiked food samples: biscuits and soya yoghourt, with a recovery range from 95 to 117 %.

Carrara [204] presented a voltammetric label-free aptasensor for detection of alpha lactalbumin (α-LB) in meal samples. The sensing strategy is based on capturing of α-LB via entrapped α-LB antibody (α-LB-Ab) through electropolymerization of polypyrrole (PPy) and then measuring the conductivity decrease by differential pulse voltammetry (DPV). A limit of detection of 0.19 fg mL^{-1} was obtained with a linear concentration range from 355 to 2840 pg mL^{-1}.

The aptasensor was applied to detect α-LB in real spiked samples of different kind of milk (UHT whole milk, low-fat milk, dry milk, and almond milk) with a recovery ranging between 93 and 97%.

An electrochemical biosensor for the detection of β-lactoglobulin was developed by Huang [205]. A DNA aptamer was used instead of an expensive antibody as the recognition element for β-lactoglobulin. The flower-like $BiVO_4$ microspheres were employed because they mimic the peroxidase catalytic activity and are able to amplify the electrochemical signal. This electrochemical biosensor exhibited a detection range from 0.01 to 1000 ng mL^{-1}, with a limit of detection of 0.007 ng mL^{-1}. The biosensor was applied to deter mine β-lactoglobulin in spiked infant food formula with a recovery ranging from 92 to 103%.

An electrochemical immunosensor based on modified screen-printed electrodes (SPEs) was designed for the detection of β-lactoglobulin (β-Lg) [206]. The surface modification of SPEs was accomplished by a simple drip coating using polyethyleneimine (PEI), reduced graphene oxide (rGO), and gold nanoclusters (AuNCs), and the obtained SPEs showed a good electrical conductivity. An anti-β-Lg antibody was then immobilized on the nanocomposite, inducing a reduction in SPEs conductivity, due to the reaction between antigen and antibody. The sensor showed a limit of detection (LOD) of 0.08 ng mL^{-1} and a detection range from 0.01 to 100 ng/mL^{-1} for β-Lg. Furthermore, milk samples from four milk brands were analyzed, and the results agreed with those from ELISA.

Finally, Abaci [207] developed a graphene oxide modified pencil graphite electrode to determine β-lactoglobulin. A linear concentration range from 0.53–11.16 mg mL^{-1} with a detection limit of 0.27 mg mL^{-1}. The sensor was applied to spiked milk samples, obtaining recoveries between 118.30–90.00%.

Peanut allergy is a frequent cause of serious anaphylactic reactions and severe diseases among food allergies.

The detection of peanut allergens in food products is sometimes challenging since they are often present unintentionally and in trace amounts or can be masked by other compounds of the food matrix [208]. Different methods are available for the peanut allergens detection, among them those based on immunoassay (ELISA) are well known and appreciated for their specificity and sensitivity, but they showed some drawbacks i.e., cross reaction, long analysis time, high cost of ELISA kits, and large numbers of sample replications. Biosensors have become attractive compared with the conventional approaches, providing real-time, possibly on-site, cost-effective, and high sensitivity analysis.

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Pingarron group developed a disposable amperometric magnetoimmunosensor for the rapid determination of Arah 2 protein, one of the major peanut allergens [208]. The approach used a sandwich configuration, involving capture and detector antibodies and carboxylic acid-modified magnetic beads (HOOC-MBs). Detector antibodies are marked with HRP-conjugated secondary antibodies and the MBs bearing the immunoconjugates are magnetically captured on surface of a disposable screen-printed carbon electrode (SPCE). The immunosensor showed a linear concentration range from 87 and 10,000 pg mL⁻with a detection limit of 26 pg mL^{-1} and a good selectivity towards possible interferent other proteins. The sensing platform was applied for the detection of Ara h 2 in different food extracts. After an appropriate sample dilution, no matrix effects were evidenced. The developed methodology determined trace amounts of the peanut allergen (0.0005%or 5.0 mg kg^{-1}) in wheat flour spiked samples and the obtained results agreed with those of ELISA kit.

Li [209]reported the synthesis of gold-palladium nanowaxberries (AuPd NWs)/dodecylamine functionalized graphene quantum dots (D-GQDs)-graphene micro-aerogel(GMA). The AuPd NWs/D-GQDs-GMA hybrid composite shows a particular three-dimensional architecture, improving significatively the amplification of detection signal.

A DNA biosensor for peanut allergen Ara h 1, based on this hybrid nanocomposite was assembled and exhibited a linearity range from 1.0 x 10^{-22} to 1.0 x 10^{-17}M with the detection limit of 4.7 x 10^{-23}M. This sensing platform was applied to the determination of peanut allergen Ara h 1 in spiked peanut milk with a corresponding recovery of 96.7%.

An innovative paper-based capacitance mast cell sensor has been designed and developed by Wang [210] for real-time monitoring of the peanut allergen Ara h 2.

A non-contact capacitance sensing platform was fabricated, employing a 3D paper chip printed with carbon electrodes. To improve the conductivity and biocompatibility of the paper chip, a polyvinyl alcohol (PVA)-gelatin methacryloyl (GelMA)-nano-hydroxyapatite (nHAP) composite hydrogel (PGHAP gel) was introduced. When rat basophilic leukemia mast cells (RBL-2H3) are immobilized and cultured on the 3D paper modified chip, signals of Ara h 2 were specifically monitored in real-time by capacitance change measurement.

A dose-dependent response for the allergen determination was obtained in the concentrations range from 0.1 to 100 ng mL^{-1}. Finally, the capacitance cell sensor performance was assessed applying it to raw peanuts and fried peanut extracts analysis. The obtained results agreed with those obtained with the conventional methods.

Previously another example of a mast cell based electrochemical sensor for the detection of different allergens in foodstuffs was developed, using the same rat basophilic leukaemia cells, and fluorescent magnetic beads [211].

Results showed that the exposure of model antigen–dinitrophenol–bovine serum albumin(DNP–BSA) to anti-DNP IgE-sensitized mast cells induced an electrochemical impedance dose-dependent signal. The detection limit was identified at 3.3 x 10^{-4} ng mL^{-1}. To demonstrate the possible application of this biosensor to real food commodities, it was employed to quantify both shrimp allergen tropomyosin (Pena1)and fish allergen parvalbumin (PV). Results show accuracy for these targets, with a limit of 0.03 µg/mL (shrimp Pena1) and 0.16ng/mL(fish PV),respectively.

A label free electrochemical immunosensor for sensitive detection of porcine serum albumin (PSA) was developed by Ahmed [212], using a stable 4-carboxyphenyl layer deposited on carbon nanofiber-modified-screen printed electrode. Antibodies were covalently attached onto the electrode. The immunosensor exhibited a linear range from 0.5 to 500 pg mL^{-1} for with detection limit of 0.5 pg mL^{-1} in buffer solution.

Cross-reactivity studies have shown good specificity with satisfactory recovery of PSA in fresh meat samples without sample dilution.

An electrochemical device using molecularly imprinted polymers (MIPs) [213] was able to detect allergenic soy marker such as genistein with a detection limit of 100ppb,

concentration known to produce adverse effect in patients. On the other hand, the sensor performance was only qualitatively validated with commercially available soy allergen detection lateral flow devices (LFDs). The MIP-sensors correctly identified the presence or absence of the genistein, with 100% accuracy in all food samples. It seems that the result of this sensor application can address a peculiar analytical challenge to achieving fast, cost-effective, and qualitative methods for direct detection of allergen tracers in food analysis.

4.Conclusion, challenges, and future perspectives

The development of highly sensitive, reliable, robust, portable, and cost-effective sensing approaches has become fundamental to guarantee the food safety, addressing the critical issue of infection/contamination of food commodities due to several causes such as bacteria, contaminants, allergens, drugs etc.

Considering the drawbacks of the conventional analytical approaches, such as complex analytical protocols, long duration of the analytical procedure, costly operation, and skilled personnel, it is quite clear that the biosensing approach is very attractive for many reasons: easy to handle, relatively low cost, good sensitivity, and easy miniaturization.

In this area, electrochemical biosensors are emerging sensing tools, especially if the combination with nanomaterials can improve the analytical performances. However, there are several issues and challenges that should be faced.

For example, some described analytical protocols involve the use of sensitive reagents and multiple-step procedures which increase measurement time and cost, making their introduction into the food safety and regulatory field very complicated.

Moreover, most of the described assays have addressed target analyte quantification just in aqueous solutions or synthetic samples (prepared by adding the contaminant in an intermediate step or even at the end of sample preparation) and only a few faces the analysis of real samples.

There are two relevant issues associated with real sample analysis: possible electrochemical interferences and efficient extraction of target analyte from the complex food matrix. To avoid electrochemical interferences, surface chemistry and type of bioreceptor need to be carefully optimized, in combination with sample pre-treatment and clean-up.

Another critical issue is connected with the bioreceptor (enzymes, antibodies, DNA, and aptamers),which represents the specificity key element for the developed sensor towards the analyte. The sensitivity of bioreceptor is limited by the immobilization protocol of the biomolecule onto the electrode surface without affecting its biological activity. Such improvements can improve the stability and overall life of biosensors.

It is worthy to evidence that, in different examples of sensors present in this review, the bioreceptor is not present at all, but it was substituted by a nanomaterial, and/or nanocomposite mimicking the bioreceptor action or activity. In this way, the issues linked to the immobilization protocols could be solved, but accurate studies and analyses of the toxicity and degradation of these nanomaterials are required.

As a general comment, nanomaterials, nanoparticles, nanocomposites, and nanostructures, used both as electrode modifiers and for electrochemical signal amplification, play an important role in the development of electrochemical biosensors for food safety with improved performance, in terms of the stability and sensitivity.

The screen-printed technology applied to electrochemical biosensors, in food quality control, provides the important features such as miniaturization of the measuring setups, a low cost of mass production, easy procedures of the use, and also the possibility of the use of such devices with small sample volumes. Further, a proper selection of nanomaterials employed for their construction can additionally improve response time, in addition to the enhancement of selectivity and sensitivity.

Sample preparation and efficient extraction of the targets remain the steady steps, limiting the total analysis time and biosensor final performance.

Moreover, more rigorous validation studies are required and the storage and operational stability of the electrochemical biosensor under real analysis conditions should be efficiently addressed and tested.

Additional work should be performed also in the application of these biosensors to the analysis of food processed samples in order to evaluate the impact of food processing on their detection capability.

Finally, the development of smart sensors is linked to the development of portable devices.

Improved portability may be achieved through the connectivity and integration of electrochemical biosensors with devices such as smartphones and tablets, but very few examples are available [129].

Such an integration of two distinct areas of research (sensors and ICT), addressing the day-to-day needs of people, could facilitate the introduction of the next generation of smart sensors into the food processing industries to increase the quality and safety of food and beverages.

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