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Electrochemical aptasensors for contaminants detection in food and environment: Recent advances

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ABSTRACT

The growing number of contaminants requires the development of new analytical tools to meet the increasing demand for legislative actions on food safety and environmental pollution control. In this context, electrochemical aptamer-based sensors appear promising among all biosensors because they permit multiplexed analysis and provide fast response, sensitivity, specificity and low cost. The aim of this review is to give the readers an overview of recent important achievements in the development of electrochemical aptamer-based biosensors for contaminant detection over the last two years. Special emphasis is placed on aptasensors based on screen-printed electrodes which show a substantial improvement of analytical performances.

Contents

1. Introduction

The development of highly sensitive and easy-to-use analytical procedures to detect contaminants (e.g. pesticides, toxins, drugs and metal ions) is utterly important in food safety and environmental analyses.

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Conventional analytical methods used in such analyses are based on chromatographic techniques, which provide sensitive and selective detection [1,2]. Despite these advantages, chromatographic techniques require highly skilled technicians for operation and they are not suitable for screening analysis. Therefore, there are continuing developments in rapid and cost-effective devices for environmental monitoring including in situ analysis [3,4]. Electrochemical biosensors are generally powerful analytical tools providing multiplexed analysis, fast response, sensitivity, specificity and low cost. The most applied electrochemical biosensors for environmental analysis use antibodies as biomolecular recognition and they are called immunosensors. Antibodies are proteins produced by an immune system in which they respond to a wide variety of agents (chemicals, drugs, bacterial toxins, etc). However, it is difficult to acquire selective, stable and sensitive antibodies characterized by low immunogenicity high toxicity and small size. Recent developments involving biomimetic synthetic receptors such as aptamers provide an interesting alternative to antibodies [5]. Aptamers are single stranded DNA or RNA short sequences, isolated in vitro from a synthetic oligonucleotide library using the automated technique, selection evolution of ligands by exponential enrichment (SELEX), first reported in 1990 [6,7]. Oligonucleotide sequences are selected from the library according to their affinity toward a target molecule to achieve the best selectivity and specificity [8]. Selected aptamers show high dissociation constants (K_d), often within nanomolar and picomolar range. In addition, their stability can be improved by chemical modifications [9]. A biosensor using aptamer as a recognition element is commonly called aptasensor. Compared to immunosensors, the use of aptasensors offers advantages such as high stability, large dynamic concentration range, prolonged shelf life and low cross reactivity [10–12]. Moreover, aptamers are easy to be modified adding functional groups, useful for their labeling and for their surface immobilization [13–15]. A growing number of aptasensors based on fluorescent, colorimetric and electrochemical methods has been proposed and their applicability to real-life sample analysis was compared to those conducted using conventional approaches [16,17]. Many reports discussed the strategies applied for small molecules binding aptamer-based biosensors realization [18,19]. In addition, some studies [20–22] showed that there were significant conformational changes associated with aptamers following their interaction with an analyte. These conformational changes were then used as recognition parameters when appropriate transducers were employed. These characteristics make the detection of small target molecules easier by enfolding them in the DNA folded structure, leading to development label free aptasensors and molecular switches [23]. Recently, a great variety of aptamers capable of binding to different targets including pesticides, toxins [4,18], antibiotics [24,25], biomarkers [26–28] was developed. Their versatility makes them particularly suitable for environmental analysis. Fig. 1 shows a distribution of electrochemical aptasensors proposed in literature in the last two years compared to other kind of aptasensors. More specifically, nearly 9% of electrochemical aptasensors for environmental and food contaminant analysis was based on screen-printed electrodes. The number of electrochemical aptasensors is continuously growing due to the ease to integrating the aptamers in the electrochemical platforms [29]. In the last few years, electrochemical aptasensors with high sensitivity were developed using nanostructured surfaces [16,30,31].

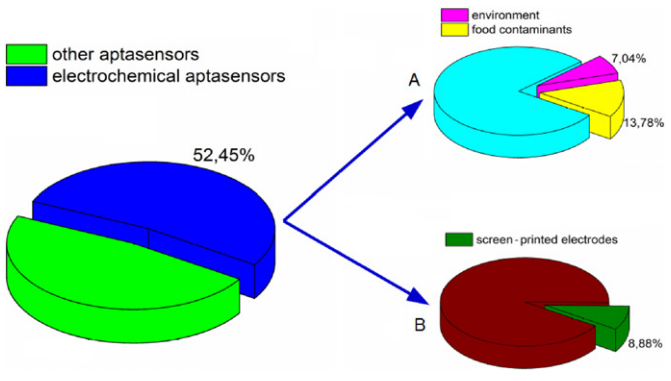

Fig. 1. Distribution of aptasensors based on electrochemical transducer and other transducer reported in literature; A) Distribution of electrochemical aptasensors for food and environmental analysis; B) Distribution of aptasensor based on screen-printed electrodes (data taken on January 2017 from scifinder using the keyword "electrochemical aptasensor" and refining the research adding the categories "environment", "food contaminants" and "screen-printed electrode").

This review provides a summary about trends and highlights in aptamer-based electrochemical sensors for contaminants analysis over the 2015–2016 period. Attention will be focused on toxic substances of heterogeneous chemical classes that contaminate food production chains, specifically, and the environment, more generally. Initially, the development of different aptasensors will be described. Recent examples of electrochemical aptasensors for various contaminants will be then presented and will be discussed in detail below for each group of molecules.

A section of the review is dedicated to aptasensors based on screen-printed electrodes (SPEs) which represent promising analytical devices for their characteristics such as cost effectiveness, reproducibility and ease of use.

2. Discussion

2.1. Aptasensor approaches

The first step in developing an aptasensor is to immobilize appropriate oligonucleotide sequences on a conducting surface. Recently, modification of the electrode surface with nanomaterials such as carbon nanotubes, graphene [32–39], metal nanoparticles [40–48], conductive polymeric layers [47,49,50] and combinations of different kind of nanostructures [50–54] has led to the development of highly sensitive aptasensors [55,56]. The immobilization step must be carried out to achieve high reactivity orientation, accessibility and stability of the surface-confined aptamer receptor and to minimize non-specific adsorption. The most successful strategies are based on covalent bond formation, affinity reactions and self-assembled layer formation [57,58]. Generally, the aptamer is functionalized with a terminal functional group such as amine, biotin and thiol group to assist the immobilization. Arm spacers such as thymidine nucleotides, tetra(ethylene-glycol) and alkyl chain are often used to preserve the analyte binding.

Regarding the assay approaches, they are largely determined by the size of the analyte and required sensitivity. Label free and label formats are proposed for aptasensors. In the label free format, the electroactivity of a redox marker at an aptasensor surface following an aptamer-analyte interaction is often monitored. In this aspect, electrochemical impedance spectroscopy (EIS) is often employed to estimate the change in electron transfer resistance following stepwise modifications at the aptasensor. Most of the developed labeled aptasensors are based on sandwich and competitive assays. The sandwich assay is used when the analyte has large size because it is able to bind more than one aptamer at the same time. In a direct sandwich assay, the first aptamer (called capture probe) is immobilized on the solid phase; the analyte is then added. After washing to remove the unreacted components, the solid phase is incubated with a secondary labeled aptamer. The amount of secondary aptamer bound to solid phase is detected.

A competitive assay is generally used when the analyte has a low molecular weight and there is, as the name indicates, a competition reaction between two reagents for a third one. In an indirect competitive assay, the solid phase is modified by the immobilization of a spacer-linked oligonucleotide sequence (the first aptamer). A complementary labeled oligonucleotide at a defined concentration and an analyte will then be allowed to compete for interacting with the immobilized aptamer. In this way, a high analyte concentration will hinder the interaction of the oligonucleotide to interact with the aptamer, leading to a smaller analytical signal.

However, in a direct competitive assay, a selected aptamer is initially immobilized on a solid phase. This is then incubated in a solution containing a defined concentration of labeled analyte and the unknown concentration of the unlabeled one. Both the labeled analyte and the unlabeled analyte compete for binding to the immobilized aptamer. An increase of the analyte concentration will cause a corresponding analytical signal decrease.

In the label formats, enzymes are the most common labels because their catalytic activity permits an increase in the limit of detection (LOD). An enzymatic product can be directly detected as electroactive species or can modify surface conductivity [47,59–67].

Recently, two labeled strategies named switch-on and switch-off are described in literature [33,35,64,67–74]. In both approaches, aptamer functionalized with electroactive molecule is immobilized on an electrode surface. In the switch-on approach, after the binding reaction with the analyte, the conformational change of the aptamer structure decreases the distance between the redox molecule and the electrode surface allowing an efficient generation of an electrochemical signal. In the switch-off strategy, there is a considerable distance between the electroactive molecule and the electrode surface, leading to a diminished electrochemical signal. Electrochemical label such as ferrocene, dyes including neutral red or methylene blue (MB) were applied in these aptasensors.

Electrochemical techniques, particularly cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), linear sweep voltammetry (LSV) potentiometry and EIS have been extensively applied in work involving aptasensors [4,32,37,38,42,52,60,75–84].

2.2. Aptasensors based on conventional electrochemical cells

Representative electrochemical aptasensors for various contaminants based on conventional electrochemical cells with different detection schemes, including their detection limit and the total measurement schedule are tabulated in Table 1. In the following sections, the aptasensors will be discussed in details.

2.2.1. Aptasensors for pesticides

A pesticide refers to any substance used to control the growth of infesting species (e.g. insect, weeds, little mammals, fungi) that can affect agricultural production. Total worldwide consumption of pesticides has been estimated around two million tons per year, about 45% of which used in Europe, 25% in USA and 25% in other countries. Pesticide application is crucial, considering that around one third of the agricultural production would be lost without it [85,86]. However, the pesticides represent some of the most toxic, stable substances released in the environment. In addition, they can have toxic effects also against non-target organisms, including humans [87].

Recently, there has been attention focusing on acetamiprid detection. Acetamiprid is a neuro-active widespread pesticide of the neonicotinoid class, synthetic derivatives of nicotine that act as a nicotinic acetylcholine receptor agonist, causing paralysis followed by death to the contaminated organism [88]. Its high toxicity was found to cause potential risk to individuals exposed to a contaminated environment [89–91]. Fei et al. [92] described a label free approach for acetamiprid detection using a nanocomposite sensor platform consisting of gold nanoparticles, multi-walled carbon nanotubes and reduced graphene oxide nanoribbons. A thiolated aptamer was chemisorbed on the gold nanoparticles. Using EIS, a LOD of 1.7×10^{-14} M acetamiprid was estimated. Similarly, Jian et al. [52] proposed an acetamiprid aptasensor made up of a silver nanoparticle decorated nitrogen doped graphene immobilized glassy carbon electrode. After attaching an aptamer to the silver nanoparticles for interaction with acetamiprid, a LOD of 3.3×10^{-14} M was estimated based on EIS. The authors then applied the aptasensor to real-life sample analysis including waste water, cucumbers and tomatoes.

2.2.2. Aptasensors for drugs

Drugs such as antibiotics, β-agonists, anti-inflammatory drugs and cocaine contaminate food and water and caused several major outbreaks worldwide. In the last few years, several aptasensors have been developed for the drugs detection [24,25]. An antibiotic designates any natural or synthetic molecules that show a toxic and selective action on single-cell organisms such as bacteria [93]. Antibiotics represent more than 70% of all consumed pharmaceuticals in veterinary medicine and they are diffused also in human medicine around 6% of all prescription [94]. A proportion of these antibiotics remains non-metabolized and can thus be spread around the environment in many ways, but principally through breeding in which antibiotics are used to accelerate animal growth (mixed with feed) in addition to therapeutic reasons. In this case, antibiotics are diffused into environment via food production or directly via excreted faeces (used also as fertilizer) and urines [94,95]. Final fate of these molecules depends on their physical-chemical properties and environmental conditions. These factors can influence their mobility, persistence and bioaccumulation [95]. One of the worst effects arose from the intake of antibiotics in human food chain is antibiotic resistance of bacteria, so they still represent one of the most challenging analytes for analytical chemistry.

Aptasensors have been developed for the antibiotic chloramphenicol used for the treatment of bacterial infections. It is only recommended to avoid the use of other antibiotics known to potentially cause lethal side effects including leukemia, aplastic anemia and grey baby syndrome [96]. Using $[Fe(CN)_6]^{3-/4-}$ as a redox probe, Pilehvar et al. [82] reported a LOD of 1.8×10^{-6} M chloramphenicol based on impedimetric measurements at a label free aptasensor. Chen et al. [40] developed an aptasensor capable of simultaneously detecting chloramphenicol and polychlorinated biphenyl-72 (an environmental contaminant). Thiolated aptamers of chloramphenicol and polychlorinated biphenyl-72 were first self-assembled on magnetic gold nanoparticles as capture probes to concentrate the target analytes. Then, their hybridized complementary oligonucleotides and a dendritic polymerase were linked to quantum dots (CdS or PbS) to form the respective nanotracers. Finally, encoded composite magnetic probes were obtained through hybridization reaction between aptamers and oligonucleotides. Once chloramphenicol and polychlorinated biphenyl-72 were recognized by the composite probes, the nanotracers were released into the supernatant where they were detected simultaneously by voltammetric measurements of the metal ion markers Cd(II) and Pb(II). LODs of 1.0×10^{-12} M chloramphenicol and 1.2×10^{-12} M polychlorinated biphenyl-72 were estimated measuring the concentration of Cd(II) and Pb(II) respectively.

Rapid analytical methods for the detection of tetracyclines are gaining interest. They are a broad-spectrum class of antibiotic of polyketide that inhibits protein synthesis and their excessive use can lead to antibiotic-resistance [95]. In this respect, multiwalled carbon nanotubes were used by Guo et al. [32] to develop a sensitive tetracycline aptasensor. In their work, a chitosan-Prussian blue-graphene modified glassy carbon electrode was applied to improve the electric conduction characteristic of the electrode and accelerate the electron transfer. The glutaraldehyde was a cross-linking agent for the immobilization of the aptamer. Using differential pulse voltammetry, they reported a LOD of 0.56×10^{-11} M tetracycline in buffered milk samples. Similarly, Shen et al. [97] reported a tetracycline signal-off aptasensor based on a Prussian blue-chitosan-glutaraldehyde modified glassy carbon electrode, which then bonded to gold nanoparticles. This then allowed NH_2 terminals of selective aptamer to interact with the gold nanoparticles. A Prussian blue-chitosan-glutaraldehyde system acting as the signal indicator was developed to improve the sensitivity of the electrochemical aptasensor. The gold nanoparticles were dropped onto the electrode to immobilize the tetracycline aptamer for preparation of the aptasensor. The tetracycline captured onto the electrode induced the current response of the electrode due to the non-conducting bimolecular. DPV measurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode yielded a LOD of 3.2×10^{-10} M tetracycline. In another work, Taghdisi et al. [98] reported

Table 1
Overview of the aptasensors based on conventional electrochemical cells for contaminants.

Analyte	Electrochemical technique	LOD [M]	Real-life tested samples	Ref.
Acetamiprid	EIS	1.7×10^{-14}	Water	[92]
	EIS	3.3×10^{-14}	Waste water, cucumber, tomato	[52]
Chloramphenicol	EIS	1.8×10^{-6}	–	[82]
	SWV	1.0×10^{-12}	Fish	[40]
	SWV	4.6×10^{-10}	Milk	[66]
Polychlorinated biphenyl-72	SWV	1.2×10^{-12}	Fish	[40]
Tetracycline	DPV	5.6×10^{-12}	Milk	[32]
	DPV	3.2×10^{-10}	Milk	[97]
	DPV	4.5×10^{-11}	Milk, serum	[98][66]
Oxytetracycline	SWV	2.2×10^{-10}	Milk	
Ampicillin	DPV	1.09×10^{-9}	Milk	[63]
	DPV	4.0×10^{-9}	Milk	[64]
Streptomycin	DPV	14.1×10^{-6}	Rat serum, milk	[60]
Kanamycin	DPV	8.7×10^{-13}	Food	[53]
	SVW	1.4×10^{-10}	Milk	[83]
	DPV	7.6×10^{-12}	Milk	[51]
	DPV	0.87×10^{-6}	Milk	[34]
	DPV	5.8×10^{-9}	Milk	[43]
	SWV	1.0×10^{-9}	Milk	[65]
	DPV	9.5×10^{-12}	Milk	[50]
Clenbuterol	EIS	1.3×10^{-12}	Pork	[110]
Salbutamol		2.2×10^{-12}		
Phenylethanolamine		7.3×10^{-12}		
Procaterol		5.96×10^{-12}		
Ractopamine	EIS	1.0×10^{-10}	Pork	[110]
	DPV	5.0×10^{-13}	Swine urine	[111]
Ibuprofen	DPV	2.0×10^{-10}	Pharmaceutical formulations, human serum, waste water	[35]
Cocaine	DPV	1.0×10^{-11}	Human serum	[42]
	DPV	1.05×10^{-11}	Rat serum	[36]
	DPV	1.3×10^{-6}	Urine	[120]
Ochratoxin-A	EIS	5.0×10^{-9}	Wine	[81]
	DPV	1.0×10^{-11}	Corn	[62]
	DPV	7.5×10^{-10}	Wine	[71]
Aflatoxin-B1	SWV	2×10^{-15}	Corn	[67]
	CV	0.1×10^{-6}	Nuts, soy sauce, wine	[49]
	EIS[a]	0.05×10^{-6}		
Fumonisin-B1	EIS	2.0×10^{-9}	Maize	[76]
Enterotoxin-B	DPV	8.5×10^{-12}	Serum	[61]
Saxitoxin	DPV	0.38×10^{-6}	Mussel	[33]
Brevetoxin-2	EIS	1.18×10^{-10}	Shellfish	[78]
Cylindrospermopsin	EIS	2.82×10^{-10}	Lake water	[37]
anatoxin-A	EIS	0.5×10^{-6}	Drinking water	[80]
	EIS	1.0×10^{-11}	Tap water	[79]
Ricin	SWV[b]	30×10^{-6}	Human serum,	[149]
		3×10^{-6}	Saliva	[72]
	SWV	–	–	
Bisphenol-A	SWV	0.6×10^{-9}	Tap water	[69]
	SWV	0.19×10^{-9}	Tap water	[70]
	DPV	2.1×10^{-11}	Environmental water	[77]
	DPV	5.0×10^{-6}	Milk	[38]
Mercury ion	DPV	0.33×10^{-6}	Tap water, river water	[158]
	DPV	0.12×10^{-9}	Lake water	[59]
	DPV	5×10^{-15}	Tap water, river water	[159]
	DPV	1.5×10^{-12}	Tap water, river water	[68]
Lead ion	CV, SWV	34.7×10^{-6}	Tap water	[160]
2, 4, 6-trinitrotoluene	SWV	4.0×10^{-6}	Soil	[164]

[a] The reported paper describes two approaches based on two different probe aptamers.
[b] The reported paper describes two approaches based on same probe aptamer.

an enzyme-amplified approach involving M-shaped complexes formed between a capture aptamer and several complementary DNA sequences, exonuclease I and a gold electrode. In the absence of tetracycline, the M-shape structure which acted as a gate and barrier for the access of $[Fe(CN)_6]^{3-/4-}$ redox probe to the surface of gold electrode remained intact, leading to a weak electrochemical signal. Upon addition of tetracycline, aptamer left complementary strands of aptamer, resulting in disassembly of M-shape structure. Subsequently, the complementary DNA strands (as ssDNAs) were digested by exonuclease I, resulting in more access of $[Fe(CN)_6]^{3-/4-}$ to the gold electrode surface and the increase of electrochemical signal. Using cyclic voltammetry, a LOD 4.5×10^{-11} M of tetracycline was achieved. The aptasensor was tested in milk (LOD 7.4×10^{-11} M) and serum (LOD 7.1×10^{-11} M) samples. Using high-capacity metal ions encoded magnetic hollow porous nanoparticles coupled with exonuclease-assisted target recycling to increase sensitivity, Yan et al. [66] developed a multiplex aptasensor for the simultaneous detection of chloramphenicol and oxytetracycline. In their work, gold nanoparticles were initially electrodeposited on a glassy carbon electrode, to which two separate DNA oligonucleotide chains that were complementary to two selected aptamers were attached via Au—S binding. The magnetic hollow porous nanoparticles were synthesized and decorated with Cd^{2+} and Pb^{2+} metal ions. Then, they were modified with complementary strands to the oligonucleotides attached on the electrode surface and applied as signal tags. First the aptamers

hybridized with the oligonucleotides immobilized forming double-strand DNA on the electrode surface. Then, with the addition of chloramphenicol and oxytetracycline, the two aptamers specifically recognized and bound the targets, resulting in the dissociation of double-strand DNA. Based on the selectivity digestion of aptamer-chloramphenicol (or aptamer-oxytetracycline) complex from exonuclease I from its 3′-end specifically, chloramphenicol and oxytetracycline were subsequently released into the solution and participated new binding with their aptamers. However, due to the terminal protection 3′-termini to 5′-end of aptamers by tethered with phosphoryl group the immobilized oligonucleotides could not be digested by exonuclease I. Therefore, after the addition signal tags, the oligonucleotides metal magnetic nanoparticles specifically hybridized with the oligonucleotides immobilized on the electrode surface. Meanwhile, the electrochemical signals of Cd^{2+} and Pb^{2+} related to the chloramphenicol and oxytetracycline concentrations significantly increased. The square wave stripping voltammetric signal of Cd^{2+} and Pb^{2+} was proportional to the concentration of chloramphenicol and oxytetracycline. The authors evaluated a LOD of 4.6×10^{-10} M for chloramphenicol and 2.2×10^{-10} M for oxytetracycline. The aptasensors were also applied to analysis of spiked milk samples.

Another broad spectrum antibiotic active against many Gram-positive and Gram-negative bacteria is ampicillin [99]. Wang et al. [63] developed an aptasensor for sensitive detection of ampicillin. The aptasensor relied on a signal-off approach with MB as a label and DPV as electrochemical technique. The aptasensor was designed by combining hairpin probe, MB-labeled probe, polymerase and nicking endonuclease. The hairpin probe contained the ampicillin-aptamer, a specific recognition sequence of nicking endonuclease and a nucleic acid segment complementary to the 5′end of the aptamer. When the aptamer recognized target ampicillin, the hairpin probe changed its conformational structure and hybridized the MB-labeled probe. This functioned as a primer to initiate an extension reaction and replicated the template part in the hairpin probe by polymerase, in which ampicillin was displaced. A new such cycle was initiated when the displaced ampicillin complexed to a second hairpin probe. Meanwhile, as a recognition sequence for nicking endonuclease was generated in the DNA duplex produced, endonuclease could nick the DNA duplex to release short single strand (ss)DNA sequences. These ssDNA sequences hybridized with MB-labeled probe which was not captured on the electrode, thus resulting in only a negligible electrochemical signal. Using DPV measurements of MB at the electrode, a LOD of 1.09×10^{-9} M ampicillin was achieved. Wang et al. [64] proposed another aptasensor based on MB-labeled hairpin probe immobilized on an indium tin oxide electrode and T7 exonuclease-assisted dual recycling signal amplification strategy. MB-labeled hairpin probe was composed of four sections: ampicillin aptamer sequences, 10 successive adenine bases used to reduce any possible steric hindrance effect, the primer recognition fragment caged in the stem of hairpin probe, and extend-DNA. When the aptamer recognized the target ampicillin, the ampicillin-aptamer complex was formed leading to the exposure of the primer recognition fragment. Then, the engaging primer annealed with the primer recognition fragment and initiated the elongation of the duplex region by polymerase in the presence of deoxynucleotides. In this way, because of the polymerase catalyzed extension of the engaging primer binding with another intact hairpin probe again, the target ampicillin was displaced and the ampicillin recycling amplification was initiated. Subsequently, the polymerization-generated partially complementary DNA duplexes recognized and digested by T7 exonuclease resulting in the release of a MB-labeled mononucleotide and the corresponding complementary strands. The released MB-labeled mononucleotide diffused toward the negatively charged ITO electrode. Thus, a distinct increase of the electrochemical signal was observed in the presence of the target. Using DPV measurements of MB at the aptasensor, a LOD of 4.0×10^{-9} M ampicillin was achieved. The aptasensor was successfully applied to assay antibiotic in milk samples.

Streptomycin is a broad-spectrum antibiotic used in human and veterinary medicine against gram-negative bacterial infections [100,101]. Its uncontrolled application may result in widespread presence in foodstuffs, which will lead to serious side effects on human health such as nephrotoxicity and ototoxicity [101,102]. Danesh et al. [60] reported a streptomycin aptasensor consisting of exonuclease I, complementary strand of aptamer, arch-shape structure of aptamer- complementary strand conjugate and a gold electrode. The hybrid was able to form a secondary arch-shape structure that inhibited electron transfer of $[Fe(CN)_6]^{3-/4-}$ redox probe at a gold electrode. Addition of streptomycin caused a conformational change and formation of streptomycin-aptamer conjugate and release of complementary strand from the aptamer. When exonuclease I was added on the electrode surface, the complementary strand (as single strand) was degraded, resulting in more access of $[Fe(CN)_6]^{3-/4-}$ to the electrode surface and the enhancement of the electrochemical signal. Using DPV measurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode, a LOD of 14.1×10^{-6} M streptomycin was estimated.

Quin et al. [53] developed an aptasensor for the detection of the aminoglycoside antibiotic kanamycin. Residues of kanamycin in food products may lead to antibiotic resistance from pathogenic bacterial strains and the molecule itself has shown side effects including nephrotoxicity and ototoxicity [103]. This aptasensor consisted of thionines functionalized graphene and a hierarchical nanoporous platinum/copper alloy as electrode surface to increase conductivity. The authors obtained a LOD of 8.7×10^{-13} M based on the differential pulse voltammetry detection of kanamycin. Kanamycin was also studied by Zhou et al. [83] that proposed a simple and label free assay based on aptamer immobilized onto a gold electrode surface. Quantitative analysis was based on the electrochemical impedance spectroscopic detection of $[Fe(CN)_6]^{3-/4-}$ redox probe at the electrode surface and a LOD of 1.4×10^{-10} M kanamycin was achieved. Guo et al. [51] used a nanoporous platinum/titanium alloy modified glassy carbon electrode for kanamycin detection. In their work, the authors immobilized the aptamer on the electrode surface modified with a film of multiwalled carbon nanotubes and the ionic liquid 1-hexyl-3-methylimidazolium hexafluorophosphate. The nanoporous platinum/titanium alloy had uniform interconnected network structure with specific surface area and was used to immobilize aptamer. After modified with the nanocomposite material, current signal was amplified due to the large specific surface area and excellent electrical conductivity of nanoporous platinum/titanium and multiwalled carbon nanotubes. Quantitative analysis was based on the DPV detection of kanamycin and a LOD of 7.6×10^{-12} M kanamycin was calculated. Similarly, Qin et al. [34] attached amino-functionalized graphene on a multiwalled carbon nanotube and 1-butyl-3-methylimidazolium hexafluorophosphate modified glassy carbon electrode, followed by an aptamer to interact with kanamycin in milk samples. Using DPV measurements, a LOD of 0.87×10^{-6} M kanamycin was estimated. An aptasensor consisting of chitosan-gold nanoparticles, graphene gold nanoparticles and multi-walled carbon nanotubes cobalt phthalocyanine nanocomposites was proposed by Sun et al. [43] for kanamycin detection. The authors immobilized a capture aptamer on the electrode before allowing it to interact with kanamycin. A signal aptamer conjugated with horseradish peroxidase (HRP) was then used to complete a sandwich format. In their work, HRP catalyzed the reduction of hydroquinone in presence of H_2O_2, upon binding with kanamycin. The reduction current measured was quantitatively related to the amount of HRP-labeled aptamer-kanamycin complex on the electrode, further related to the concentration of kanamycin. Using amperometric measurements of H_2O_2, a LOD of 5.8×10^{-9} M kanamycin was estimated. Xu et al. [65] developed aptasensor for kanamycin based on label free and labeled signal-on square wave voltammetric measurements. A DNA sequence was initially immobilized on a gold electrode, which then hybridized with a complementary aptamer sequence. The formed duplex DNA has 3′ hydroxyl blunt termini as a recognition element, and DNA complementary could be stepwise removed by exonuclease III ultimately resulting in the release of the kanamycin-aptamer for successive new cycles of hybridization and cleavage. Upon sensing the analyte, kanamycin-aptamer complex inhibited the cleavage process catalyzed by exonuclease III.

In this way, the concentration of the retained DNA sequence was proportional to that of kanamycin, which was quantified by SWV measurements of $[Ru(NH_3)_6]^{3+}$ at the electrode, obtaining a LOD of 1.0×10^{-9} M kanamycin. The same research group also proposed a sandwich approach by assembling graphene-polyaniline and polyamidoamine dendrimer gold nanoparticle (PAMAM–Au) nanocomposites on the surface of a glassy carbon electrode [50]. The target analyte was first captured on the modified electrode surface. Then, sandwich assay was completed using a secondary aptamer labeled with biotin on which HRP was linked via streptavidin-biotin interaction. Current given from electro-reduction reaction of H_2O_2 catalyzed by HRP was recorded via DPV obtaining a LOD of 9.5×10^{-12} M kanamycin.

As well as antibiotics, indiscriminate diffusion and bioaccumulation of the other drugs present potential hazard for environmental health [104–107]. Aptasensors based on label free approaches were described by Chen et al. for molecules acting as β-agonists (e.g. ractopamine, clenbuterol, salbutamol, phenylethanolamine and procaterol) that can accumulate in body tissues and cause acute or chronic poisoning in case of overuse of these drugs [108,109]. EIS measurements of $[Fe(CN)_6]^{3/4-}$ were performed at aptamer-modified gold electrodes to study the binding of aptamers and targeted analytes [110]. The obtained LODs were 1.0×10^{-10} M ractopamine, 1.3×10^{-12} M clenbuterol, 2.2×10^{-12} M salbutamol, 7.3×10^{-12} M phenylethanolamine and 5.96×10^{-12} M procaterol. Yang et al. [111] also studied ractopamine detection at a gold nanoparticle/poly-dimethyl-diallyl amine-graphene nanocomposite based electrode. The aptamer was attached on AuNPs of the composite membrane via Au—S binding. Differential pulse voltammetry of $[Fe(CN)_6]^{3/4-}$ from a signal-off assay at this electrode yielded a LOD of 5.0×10^{-13} M.

Ibuprofen is a nonsteroidal anti-inflammatory drug commonly used for relieving pain (even after surgery), for alleviating fever and in case of inflammatory diseases. A long term overuse of this drug could double the risk of heart attack [112,113] and even other toxic effects [114]. Roushani et al. [35] developed a multiwalled carbon nanotube/ionic liquid/chitosan modified glassy carbon electrode label-based aptasensor for the detection of ibuprofen. The ionic liquid used for the electrode modification was 1-methyl-3-octyl imidazolium tetra fluoro borate. The amino groups of chitosan in the nanocomposite were used for the covalent immobilization of the amine-capture probe (ssDNA1) via terephthalaldehyde as the linking agent. The addition of the ibuprofen specific aptamer (ssDNA2) as a biorecognition element led to the formation of a half-duplex DNA structure through the hybridization between the ssDNA1 and ssDNA2. MB redox probe intercalated the hybrids formed on the electrode surface and the transduction principle was based on its electron transfer. Upon the incubation of the target ibuprofen, the peak currents of MB measured by DPV experiments decreased due to the formation of the aptamer-ibuprofen complex and the displacement of MB from the DNA. It was found that the aptasensor detected the ibuprofen with a linear range (70×10^{-9} M up to 6×10^{-3} M) and the detection limit as low as 2×10^{-10} M. The aptasensor was also tested in real samples, spiking pharmaceutical formulations, human serum and waste water.

Finally, we also reported electrochemical aptasensors for cocaine detection. This analyte and its metabolites are emerging pollutants. The residues appear to be widespread at very low concentrations in all populate zones [115]. Cocaine is a drug which abuse causes physiological and psychosocial health problems. It can be a contaminant because its diffusion in the environment [116–119]. Roushani et al. [42] developed an aptasensor for cocaine by immobilizing a selected aptamer on a multiwalled carbon nanotubes/ionic liquid/chitosan nanocomposite modified glassy carbon electrode that was subsequently coated with gold nanoparticles. DPV measurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode was used to quantitatively relate to cocaine. In their work, a LOD of 1.0×10^{-11} M was estimated. Taghdisi et al. [36] described an aptasensor for the detection of cocaine. The assay was based on aptamer already attached to a complementary single-strand DNA immobilized on a gold electrode. In the presence of the analyte, the interaction of the analyte and aptamer would cleave the aptamer-DNA bond, allowing the single-strand DNA to bind to single-walled carbon nanotubes. Because the aptamers bound to their targets with a greater binding constant compared to aptamers binding to their complimentary strands, the addition of single-walled carbon nanotubes on the surface of electrode, led to the strong binding of single-walled carbon nanotubes and complementary single-strand DNA through π–π interactions. Quantitative determination was based on DPV detection of cocaine and a LOD of 1.05×10^{-11} M was estimated. An enzyme-based approach, presented by Shen et al. [120], exploited the use of a supramolecular aptamer, rolling circle DNA amplification and multiple binding of the enzyme alkaline-phosphatase. In their work, a selective cocaine aptasensor was designed cleaving the selected aptamer in two fragments: one fragment was immobilized on a gold electrode, and the second fragment was labeled with biotin at its 3′-end before being conjugated streptavidin. In the presence of cocaine, the two strands form a supramolecular structure that conjugates to streptavidin for anchoring of biotinylated circular DNA in the presence of cocaine. Using phi29 DNA polymerase (from *Bacillus phage phi29*), it was possible to produce micrometer-long single-strand. DNA containing hundreds of tandem repeats could bind to many biotinylated detection probes. Streptavidin-alkaline phosphatase conjugate was then added to trace the affinity reaction. The enzyme catalyzed the hydrolysis of 1-naphthyl phosphate to 1-naphthol. The enzymatic product was detected by DPV, obtaining a LOD of 1.3×10^{-6} M.

2.2.3. Aptasensors for toxins

Mycotoxins represent the most diffused group of toxins that affect food products. They are secondary metabolites of filamentous fungi and many different substances produced by different mycotoxigenic species form them. The infection of agricultural crops can occur at any production stage: during crop growth, harvest, storage or processing [121]. They showed several adverse impacts on human health, such as gastrointestinal diseases, kidney damage and immune suppression, in general defined as mycotoxicosis. The severity of mycotoxicosis depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed. The chemical structures of mycotoxins are various, but they are all relatively low molecular mass compounds [122].

Ochratoxin-A (OTA) is a low molecular weight mycotoxin which is characterized by high toxicity at very low concentration. The European Food Safety Authority (EFSA) established the tolerable weekly intake of ochratoxin-A (on advice of the Scientific Panel on Contaminants in the Food Chain) at 120 ng/kg. It was suggested to contribute to the development of cancer by the International Agency for Research on Cancer; in addition, it was considered nephrotoxic, teratogenic and immunosuppressive [123,124]. A label free approach for OTA was reported by Mejri-Omrani et al. [81]. The aptasensor was based on a polypyrrole/polyamidoamine dendrimer coated gold electrode, to which a selected aptamer was covalently attached. The interaction of this aptamer with OTA was evaluated by EIS measurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode and a LOD of 5.0×10^{-9} M was achieved. In the work of Tan et al. [62], a competitive assay between a methylene blue conjugated DNA probe for OTA and free OTA was conducted at an OTA aptamer-modified indium/tin oxide (ITO) electrode. MB-labeled probe DNA was hybridized with the OTA aptamer. The hybrid formed could not diffuse to the negative charged ITO electrode surface due to the repulsion of the negative charges, In the presence of target OTA, the aptamer preferred to form an OTA-aptamer complex in lieu of an aptamer-DNA duplex, which resulted in the dissociation of probe DNA from the probe DNA-aptamer complex. The released probe DNA was then digested into mononucleotides, including MB-labeled electroactive ones (eT), from $recJ_f$ exonuclease (a single-stranded DNA specific exonuclease which catalyzes the removal of deoxy-nucleotidemonophosphates from DNA in the 5′ → 3′ direction), increasing electrochemical signal due to free MB diffusion to the negative charged ITO electrode surface. Using

DPV measurements of MB at the electrode, a LOD of 1.0×10^{-11} M was obtained and corn samples were also tested. Yang et al. [71] developed an aptasensor in which a capture DNA oligonucleotide was immobilized on a gold electrode surface. The oligonucleotide sequence was able to bind an OTA aptamer forming a double strand structure with reporter DNA bound gold nanoparticles. A guanine-rich DNA oligonucleotide was also able to bind the unoccupied chains on the nanoparticles surface. Increasing the target concentration, a decrease of labeled nanoparticles bound was obtained. MB was detected via DPV and a LOD of 7.5×10^{-10} M was obtained.

Aflatoxin-B1 is a strongly toxic metabolite produced by several molds, specially diffused in crops [125]. It was described as a carcinogenic substance (first hazard class in accordance with the classification of the International Agency for Research on Cancer) [126,127]. Zheng et al. [67] reported an aptasensor for aflatoxin-B1 detection by adopting a telomerase and exonuclease III based two-round signal amplification strategy. A DNA oligonucleotide was immobilized on Au-NPs, which would then interact with a complementary aptamer on a gold electrode. Telomerase was used to elongate unbound DNA oligonucleotide sequence. The analyte competed for the binding site. MB was then added as electrochemical mediator. Exonuclease III was used to hydrolyze the 3′-end of the aptamer-target adduct, causing the release of bound aflatoxin-B1, available for the following recognition-sensing cycle. Using SWV measurements of MB a LOD of 2×10^{-15} M aflatoxin-B1 was obtained. Evtugyn et al. [49] developed an assay for aflatoxin-B1 detection based on a modified glassy carbon electrode. Neutral red and polycarboxylated macrocyclic ligands were electropolymerized for covalent aptamer immobilization. Interaction with the analyte was evaluated using both cyclic and EIS obtaining a LOD of 0.1×10^{-6} M and 0.05×10^{-6} M respectively.

Fumonisin B1 is the most prevalent member of a family of toxins, known as fumonisins, produced by several species of Fusarium molds, such as *Fusarium verticillioides*. It is mostly found in maize, in processed maize products and animal feeds [128,129]. Its detection remains important because it is considered a possible carcinogen by the International Agency for Research on Cancer, being related with esophageal and liver cancers in some countries [130–132]. Chen et al. [76] described a simple label free impedimetric aptasensor based on a gold nanoparticle modified glassy carbon electrode. A Fumonisin B1 aptamer was immobilized via Au—S interaction. EIS measurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode was used to detect Fumonisin B1 and a LOD of 2.0×10^{-9} M was estimated.

Staphylococcal enterotoxin-B is one of the most epidemic antigens that can cause serious immunosuppression and give lethal effects even at low concentration [133,134]. Deng et al. [61] described an aptamer-based sensor for its detection based on a gold electrode, modified with a nanocomposite adduct of gold nanoparticles, zirconia nanoparticles, and chitosan. A DNA oligonucleotide sequence was immobilized onto the modified surface in order to bind the selected aptamer for staphylococcal enterotoxin-B. In the presence of the analyte, the aptamer is released and the immobilized DNA sequence was bound using a biotinylated oligonucleotide added as detection probe. Streptavidin-HRP was then bound to the biotinylated DNA and reduction peak currents of H_2O_2 were measured by DPV obtaining a LOD of 8.5×10^{-12} M.

Algal toxins (mostly cyanobacterial toxins produced from blue green algae) represent another group on which interest is continuously growing. Massive discharges of nutrients (mainly from agricultural wastewater) have led to increased algal bloom and toxins produced by these algae were implicated adverse effects against sea wildlife and also humans. Most common algal toxins are microcystins, nodularins, anatoxins, cylindrospermopsin, and saxitoxins and toxic effects can affect liver and nervous system. All the countries using surface water as a drinking water source have to face up with contamination given by these compounds [107].

Saxitoxin, one of the most harmful marine toxins, and its analogues, such as neosaxitoxin and gonyautoxins, are potent neurotoxins that can block mammalian voltage-gated sodium channels and result in death [135,136]. Saxitoxin can enter the marine food chain, causing the "paralytic shellfish poisoning" via contaminated seafood or water intake [137,138]. Hou et al. [33] developed an aptasensor based on signal-off strategy for the detection of saxitoxin. A gold electrode was modified with carbon nanotubes containing electrostatically anchored MB. The aptamer was bound on the previously described self-assembled monolayer and its interaction with the analyte was evaluated using oxidation peak current of MB by DPV obtaining a LOD of 0.38×10^{-6} M saxitoxin. It was also tested on mussel samples.

Brevetoxins are potent cyclic polyether neurotoxins produced by the marine red tide dinoflagellates *karenia brevis* and accumulate in shellfish. Human exposure to these substances can occur through food (contaminated shellfish) and even trough aerosol exposure in marine zones. Brevetoxins are neurotoxic and can cause mortality [139,140]. Eissa et al. [78] reported a label free aptamer-based competitive approach by covalently immobilizing brevetoxin-2 on a gold electrode surface. Solutions containing the selected aptamer and different concentrations of brevetoxin-2 were then incubated onto the modified surface. The immobilized analyte interacted with free aptamer; the amount of bound aptamer at the electrode surface was evaluated by EIS measurements of $[Fe(CN)_6]^{3-/4-}$. These authors estimated a LOD of 1.18×10^{-10} M brevetoxin-2.

Cylindrospermopsin is one of the most hazardous cyanotoxins in freshwater sources, showing strong cytotoxicity, developmental toxicity and carcinogenic activity; its assumption can also bring to multiple organ dysfunction syndrome [141–144]. Zhao et al. [37] developed a label free impedimetric aptasensor for cylindrospermopsin by covalently grafting the aptamer on a thionine/graphene-modified glassy carbon electrode using glutaraldehyde as cross linker. The sensor was able to capture the analyte from solutions. EIS masurements of $[Fe(CN)_6]^{3-/4-}$ at the electrode were used to detect cylindrospermopsin and a LOD of 2.82×10^{-10} M was estimated. Elshafey et al. [80] developed an impedimetric aptasensor for anatoxin-A, another compound of the cyanotoxins class, which is found to contaminate drinking water [145,146]. By exploiting an Au—S interaction, they immobilized a thiolated aptamer on a gold electrode. Using $[Fe(CN)_6]^{3-/4-}$ solution for EIS measurements, a proportional % reduction of electron transfer resistance was observed in different concentrations of anatoxin-A. These authors evaluated a LOD of 0.5×10^{-6} M anatoxin-A. Elshafey et al. [79] also proposed an impedimetric aptasensor for anatoxin-A, starting from the selection of the high affinity aptamer that has subsequently immobilized on a gold electrode surface. Using $[Fe(CN)_6]^{3-/4-}$ solution for EIS measurements, a LOD of 1.0×10^{-11} M anatoxin-A was obtained.

Ricin is a toxin derived from the castor bean plant *Ricinus communis*. Poisoning can occur via ingestion, inhalation, or injection and can have symptoms similar to gastroenteritis or respiratory illnesses. It is known to kill eukaryotic cells by inactivating ribosomes and blocking protein synthesis [147,148]. Du et al. [149] described a ricin aptasensor based on a kinetic competitive approach. The authors mixed an aptamer that was previously bound to ricin with a MB-labeled blocker and a molecular beacon. To the extent that the target occupied the aptamer, the MB-labeled blocker failed to bind to the aptamer but, instead, bound to the molecular beacon. The kinetic competition did not work if the target and blocker were allowed to equilibrate with the aptamer in the absence of the molecular beacon. Even in this case, the assay worked as a signal-on sensor. In the presence of analyte, more MB-labeled blocker remained in solution, which in turn allowed the blocker to interact with the MB on the electrode surface, producing an increased electrochemical signal. Using SWV signals of the MB a LOD of 3×10^{-6} M ricin toxin was calculated.

Fetter et al. [72] reported a simple strategy for the detection of ricin and botulinum toxins. Botulinum is a bacterial neurotoxin common in soil and water causing a potential fatal disease named botulism [150]. The aptasensor was based on a gold electrode on which surface MB-labeled aptamers for ricin and botulinum were respectively immobilized

using Au—S interaction. Interactions between the aptamers and the targets induced aptamer conformation changes that, due to the altered position and dynamics of the electrochemically active moiety, caused characteristic changes in the observed current output by SWV measurements of MB at the electrode.

2.2.4. Aptasensors for endocrine disruptors

Endocrine disruptors are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. A wide range of substances, both natural and synthetic, are responsible for endocrine disruption. Several examples of aptasensors for endocrine disrupting compounds analysis are described in literature to illustrate the usefulness of the biosensor technology for environmental applications. Bisphenol-A is a diffused industrial compound involved in the production of polycarbonate plastics and epoxy resins. It can be found in many plastic consumer products, it is non-biodegradable and highly resistant to chemical degradation [151,152]. It is a well-known and much studied contaminant causing a wide range of health problems to living beings, especially the young [152,153]. Some bisphenol-A aptasensors have been developed over the last years. Liu et al. [59] reported a signal-off aptasensor based on a gold-modified glassy carbon electrode. In their work, bisphenol-A and a MB-labeled complementary DNA oligonucleotide competed for a DNA probe on the electrode. Quantitative analysis was based on SWV detection of MB, a LOD of 0.6×10^{-9} M bisphenol-A was obtained. Real-life samples of tap water were also analyzed. Yu et al. [70] described a competitive aptasensor. The aptamer was immobilized on a gold electrode surface by chemisorption. The aptamer was labeled with ferrocene and its complementary oligonucleotide sequence, labeled with MB. The complementary sequence and the analyte competed for the immobilized aptamer. The two labels acted as a dual amplification system. An increase in bisphenol-A concentration yielded a lower signal from the MB-labeled complementary DNA probe and a higher signal from the ferrocene-labeled aptamer. The specific interaction between bisphenol-A and ferrocene-labeled aptamer led to the release of MB-labeled complementary DNA probe from the sensing interface and the conformational change of ferrocene-labeled aptamer. As a result, the oxidation peak currents of ferrocene and bisphenol-A increased with the increase of the concentration of target according to the signal-on mode while that of MB decreased with the increase of the bisphenol-A concentration according to the signal-off mode. Using SWV, a LOD of 0.19×10^{-9} M bisphenol-A was estimated. Moreover, real-life sample analysis was carried out on tap water samples. Derikvand et al. [77] described an aptasensor based on label free approach. Highly dispersed platinum nanoparticles on acid-oxidized carbon nanotubes and functionalized with polyethyleneimine were deposited on a glassy carbon electrode surface. An ammine-capped DNA oligonucleotide was bound to the carboxyl groups on the nanostructured surface, in order to bind the aptamer forming a double strand structure. A complementary bisphenol-A–aptamer as a detection probe to hybridize with the immobilized oligonucleotide was designed. By adding bisphenol-A, the aptamer specifically bound to bisphenol-A and its end folded into a bisphenol-A binding junction. Because of steric/conformational restrictions caused by bisphenol-A aptamer–complex formation at the surface of modified electrode, the interfacial electron transfer resistance of $[Fe(CN)_6]^{3-/4-}$ was blocked. Sensitive quantitative detection of bisphenol-A was carried out by monitoring the decrease of differential pulse voltammetric responses of $[Fe(CN)_6]^{3-/4-}$ peak currents with increasing bisphenol-A concentrations. A LOD of 2.1×10^{-11} M bisphenol-A was evaluated. The aptamer-based assay realized by the authors is shown in Fig. 2. Zhou et al. [38] reported a label-free bisphenol-A aptasensor based on a glassy carbon electrode modified with gold-dotted graphene. Selected aptamer was immobilized on the electrode surface by S—Au binding. The interaction between bisphenol-A aptamer and target was investigated by the electrochemical probe of $[Fe(CN)_6]^{3-/4-}$ and monitored by CV and DPV. The authors estimated a LOD of 5.0×10^{-6} M bisphenol-A.

2.2.5. Aptasensors for heavy metals

Heavy metals are an important class of pollutants with both lethal and sub-lethal effects on organisms, because most of them can be toxic to all forms of life at high concentrations due to formation of complex compounds within the cell. An essential difference compared to organic pollutants is the fact that heavy metals introduced into the environment cannot be biodegraded, but they persist indefinitely and accumulate in air, water and soil. Industrial growth has led to a massive diffusion of these pollutants, sometimes determining high exposure even through dietary intake of plant-derived food and beverages, drinking water and air. Heavy metal ions have long-term effects on human health, ranging from nervous system damage to renal dysfunction or lung disease and respiratory system damages. In some cases, they are related to lung cancer [154,155]. For these reasons, they are detected to evaluate environmental pollution. Mercury ions (Hg^{2+}) are reported as one of the most toxic heavy metal ions. The exposure to mercury can lead to a series of harmful effects to health, such as brain damage, kidney failure, nervous system damage and immune system damage [156,157]. Wu et al. [158] reported an aptasensor for Hg^{2+} detection that consisted of streptavidin modified magnetic beads on a magnetic glassy carbon electrode, to which a biotin-labeled thymine-rich DNA aptamer was attached. This electrode was used to capture Hg^{2+} to form a secondary structure with intercalated thionine acting as a signal mediator. Using DPV, the authors estimated a LOD of 0.33×10^{-6} M Hg^{2+}. Bao et al. [59] reported an enzyme-based approach for mercury ions detection. An oligonucleotide capture probe was immobilized on a gold electrode surface and was able to bind the complementary thymine-rich aptamer sequence. Its interaction with the analyte permits exonuclease III to digest the double strand-like structure containing Hg^{2+}, subsequently available for a recycle. With each digestion cycle, a digestion product named as help DNA which had partially complementary segments with capture probe and two oligonucleotides used as auxiliary DNA. The presence of help DNA and two auxiliary DNA collectively facilitated successful hybridization chain reaction process and formed long double-stranded DNA. The $[Ru(NH_3)_6]^{3+}$ electrochemical mediator was electrostatically absorbed on the double-strand DNA. DPV current increased with the increasing concentration of Hg^{2+}. Higher concentration of Hg^{2+} led to larger amount of help DNA, which played an important role in assisting the next hybridization chain reaction amplification. Using DPV, the authors evaluated a LOD of 0.12×10^{-9} M Hg^{2+}. Li et al. [159] developed an aptasensor for Hg^{2+} detection based on a self-assembly of thiolated β-cyclodextrins on a gold electrode. Orderly arranged molecular layer showed interspaces among β-cyclodextrins that allowed the electrochemical probe $[Fe(CN)_6]^{3-/4-}$ to reach electrode surface. Thionine labeled aptamer was then linked to β-cyclodextrins and hybridized with a DNA oligonucleotide, generating free interspaces. Interaction with the analyte brought to the formation of a secondary structure that closed interspaces, lowering the signal. Quantitative analysis was based on DPV detection of $[Fe(CN)_6]^{3-/4-}$ and a LOD of 5.0×10^{-15} M Hg^{2+} was achieved. The aptamer-based assay is shown in Fig. 3. The biosensor reported by Gao et al. [68] was based on a thiolated aptamer with 22 thymine bases immobilized on a gold electrode surface by S—Au bonds. When Hg^{2+} was absent in the test solution, aptamer showed a single stranded and random coil structure, and neutral red could not interact with aptamer. As a result, no electrochemical response was observed on the sensor. However, when the Hg^{2+} ions were present and captured by the aptamer through tymine-Hg^{2+}-thymine coordination chemistry, the random coil structured aptamer on the electrode surface was transformed to the folded hairpin-like double helix structure. This secondary structure of aptamer-target complex was able to bind to the electron mediator neutral red. Using DPV the electrochemical signal of neutral red at the electrode were measured and a LOD of 1.5×10^{-12} M Hg^{2+} was achieved.

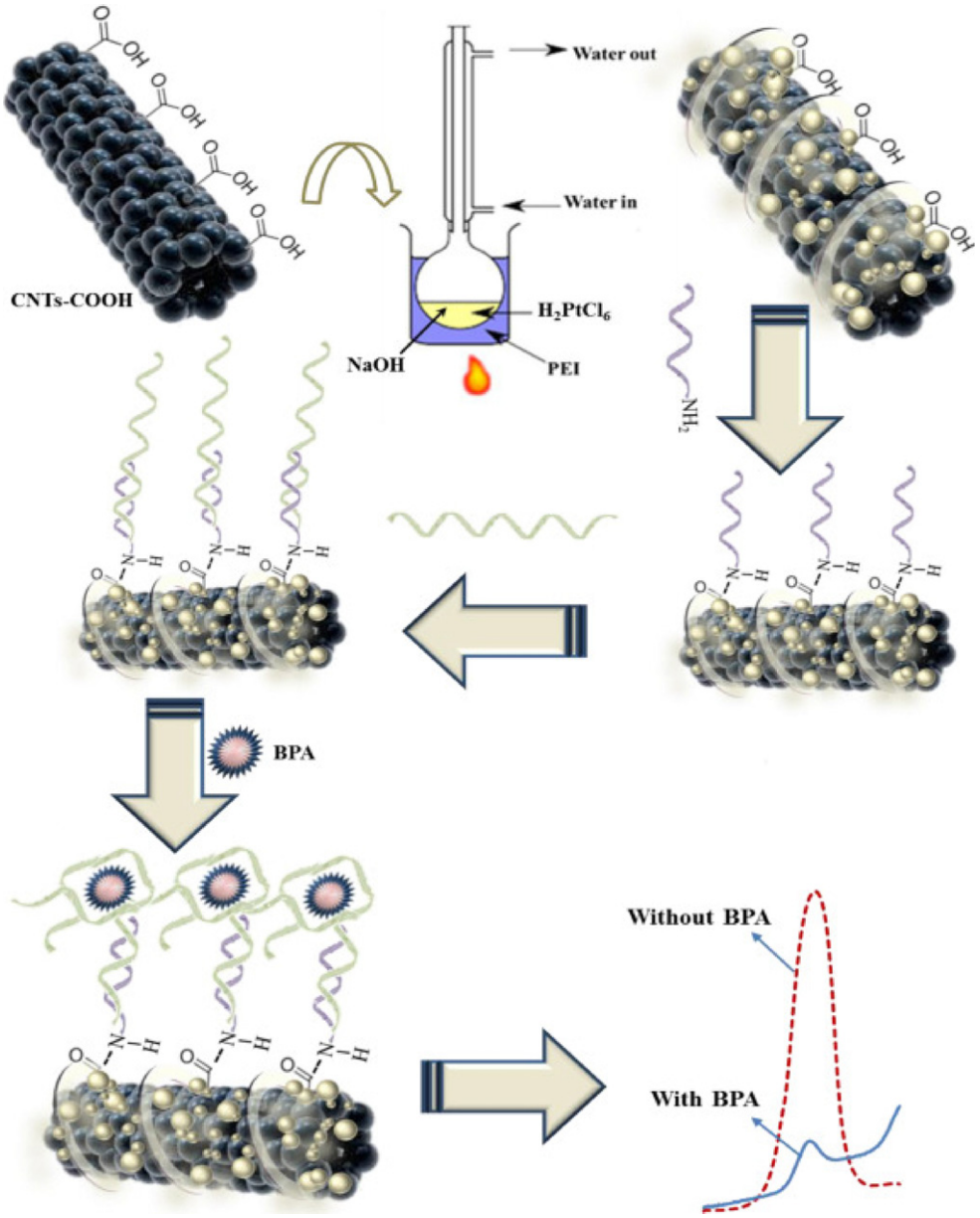

Fig. 2. Scheme of the aptasensor for bisphenol-A detection proposed from Derikvand et al. [77]. Highly dispersed Pt nanoparticles/acid-oxidized carbon nanotubes, functionalized with polyethyleneimine were deposited on a glassy carbon electrode. An ammine-capped DNA oligonucleotide, complementary to the specific aptamer, was bound to the nanostructured surface, in order to bind the aptamer forming a double strand structure. DPV measurements, using $[Fe(CN)_6]^{3-/4-}$ as electrochemical mediator were carried out, observing a decrease of the signal in presence of the analyte. Reprinted with permission of Elsevier.

Using Au—S interaction, Jarczewka et al. [160] immobilized a short oligonucleotide DNA probe on a gold electrode to develop an aptasensor for Pb(II) detection. Binding of Pb(II) ions was followed by formation of G-quadruplex structures (confirmed by EIS measurements), while MB was used in voltammetric measurements (CV and SWV) for analytical signal generation. Due to the stabilization of G-quadruplexes by lead ions, the oligonucleotide strand folding occurred and, as a consequence, enhancement of electron transfer resistance between MB and electrode surface was observed. MB current signals increased proportionally with Pb(II) ion concentration in the range from 0.05 to 1×10^{-3} M and the estimated LOD was 34.7×10^{-6} M. Pb(II) determination in real-life water samples was also conducted.

2.2.6. Aptasensors for other contaminants

The molecule 2,4,6-trinitrotoluene is usually not considered a pollutant but its extensive use has led to contamination of soil and groundwater [161,162]. The Environmental Protection Agency considers 2,4,6-trinitrotoluene toxic because it shows diffused carcinogenic and mutagenic effects [162,163]. Shorie et al. [164] presented an approach for selection and utilization specific aptamers for detection of explosives including 2,4,6-trinitrotoluene. Following the immobilization of the aptamer on a gold electrode surface, the reduction current of the nitro group of 2,4,6-trinitrotoluene was used to relate quantitatively to 2,4,6-trinitrotoluene. A LOD of 4.0×10^{-6} M was obtained in Tris buffer.

2.3. New aptasensor approaches based on screen-printed electrodes

Conventional electrodes are now being replaced by screen-printed electrodes (SPEs) connected to miniaturized potentiostat in order to develop and to apply friendly procedures for environmental analysis. Screen-printing technology permits design flexibility, process automation, good reproducibility and a wide choice of materials for electrode surface construction [165]. SPEs have numerous advantages in comparison to conventional electrodes such as low cost, easy production, small

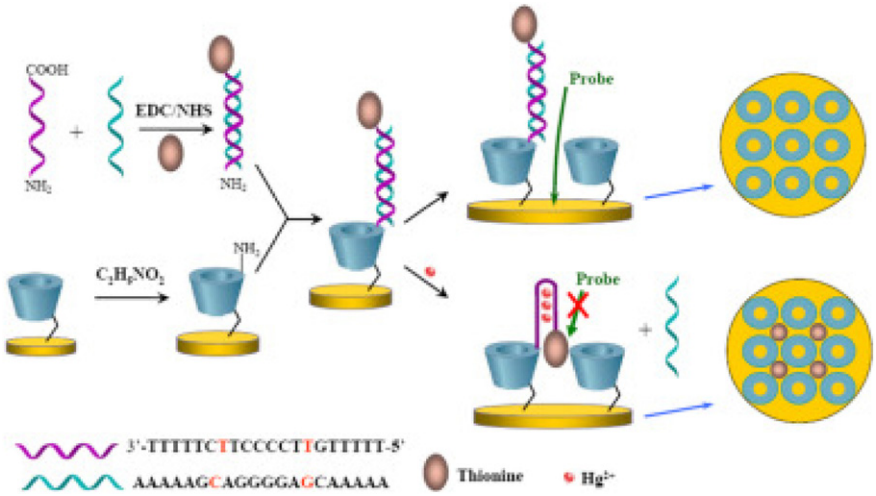

Fig. 3. Scheme of the aptasensor for Hg^{2+} detection based on a self-assembly of thiolated β-cyclodextrins on a gold electrode [159]. Orderly arranged molecularly layer showed interspaces among β-cyclodextrins that let electrochemical probe $[Fe(CN)_6]^{3-/4-}$ to reach electrode surface. Thionine labeled aptamer was then linked to β-cyclodextrins and hybridized with a DNA oligonucleotide, letting interspaces free. Interaction with the target brought to the formation of a secondary structure that closed interspaces, lowering the DPV signal. Reprinted with permission of Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dimensions, enabling the development of pocket-size devices that suit field analysis and decentralized laboratories where treatments for toxic and hazardous wastes are not available [166–168]. Single use of SPEs has avoided electrode fouling and negated cleaning procedures that could affect measurement reproducibility [169]. However, there are limitations when SPEs are used in analysis involving organic solvents that can dissolute the insulating printing inks [170], although there are examples of organic solvent-compatible SPEs [171]. Despite that unmodified SPEs were used in contaminant analysis [172], many electrochemical and (bio)chemical modifications are usually applied to increase their analytical performances [165,169]. More recently, SPEs coupled with nanomaterials for increasing biosensor sensitivity were extensively described [31,56,173].

Screen-printed aptasensors are reported for the class of contaminants described in previous sections. The representative electrochemical aptasensors based on SPEs with different detection approaches, including their detection limit and the total measurement schedule are tabulated in Table 2.

2.3.1. Aptasensors for pesticides

Rapini et al. [47] reported a nanostructured aptasensor for acetamiprid detection. The aptamer was immobilized on a polyaniline gold nanoparticle modified graphite SPE. Acetamiprid and a biotinylated DNA oligonucleotide were then allowed to competition binding to the aptamer. Streptavidin-labeled alkaline phosphatase was then bound to the biotinylated oligonucleotide, so that the enzyme would catalyze the conversion of 1-naphthyl phosphate to 1-naphthol. In this way, the oxidation current of 1-naphthol was used to quantitatively relate to acetamiprid. Using DPV, a LOD of 86×10^{-6} M acetamiprid was achieved. Real-life sample analysis involving spiked fruit juices was also carried out and compared with chromatographic measurements.

Table 2
Overview of the aptasensors based on screen-printed electrodes for contaminants.

Analyte	Electrochemical technique	LOD [M]	Real-life tested samples	Ref.
Acetamiprid	DPV	86×10^{-6}	Fruit juices	[47]
Chloramphenicol	DPV	4.0×10^{-6}	Human serum	[44]
	SWV	4.0×10^{-6}	Human serum	[45]
	DPV	1.83×10^{-10}	Milk	[174]
	DPV	6×10^{-6}	–	[54]
Ochratoxin-A	EIS	14×10^{-9}	Wine	[48]
	DPV	2×10^{-11}	Cocoa	[73]
	EIS	3.7×10^{-10}	Cocoa beans	[57]
	DPV	2×10^{-10}	Cocoa beans	[175]
	CV	0.1×10^{-6}	Corn	[46]
	DPV	1.4×10^{-11}	Wheat	[39]
Aflatoxin-M1	EIS	3.5×10^{-12}	Milk	[84]
Aflatoxin-B1	EIS[a]	3.8×10^{-10}	Beer	[58]
		8.0×10^{-10}	Wine	
Toxin-A	CV	1×10^{-6}	–	[41]
Arsenic	DPV	0.15×10^{-6}	Tap water, lake water	[74]

[a] The reported paper describes two approaches based on two different probe aptamers.

2.3.2. Aptasensors for drugs

Bagheri Hashkavayi et al. [45] described an electrochemical aptasensor for chloramphenicol detection using a gold nanocube and cysteine-modified gold SPE. The aptamer was immobilized on the nanostructured surface and its interaction with chloramphenicol was measured by SWV. The reduction peak of chloramphenicol at the electrode was measured obtaining a LOD of 4.0×10^{-6} M. The same authors subsequently reported another aptasensor for chloramphenicol detection, based on graphite SPE that was modified with a dendritic nanostructure based on mesoporous silica functionalized with 1,4-diazabicyclo [2,2,2]octane in order to increase gold nanoparticles immobilization [44]. The selected aptamer was then bound on gold nanoparticles. The analyte was detected via DPV, using hemin as electrochemical indicator. A LOD of 4.0×10^{-6} M was achieved. The aptamer-based assay developed is shown in Fig. 4. Hamidi-Asl et al. [174] presented another SPE--based aptasensor for chloramphenicol detection, combining the aptamer with different type-B gelatins, before applying it on a gold SPE surface. Based on the chloramphenicol reduction at the SPE a LOD of 1.83×10^{-10} M was achieved. Zhan et al. [54] constructed a tetracycline aptasensor based on a reduced graphene oxide/magnetite (Fe_3O_4)/sodium alginate composite modified graphite SPE. The oxidation peak current of tetracycline obtained by DPV in detection buffer (acetate buffer containing thionine), increased significantly with the increasing concentration tetracycline and a LOD of 6×10^{-6} M was achieved.

2.3.3. Aptasensors for toxins

Rivas et al. [48] reported a label free impedimetric OTA aptasensor. The aptasensor was prepared modifying the electrode surface by electropolymerization of thionine and adsorption of IrO_2 nanoparticles. The aptamer was then immobilized by electrostatic interactions produced by the attraction between the negatively charged citrate groups surrounding IrO_2 nanoparticles and the positively charged amino-groups of the amino-modified aptamer at neutral pH. Hydrophobic forces were also involved in the aptamer–IrO_2 nanoparticle interaction, contributing

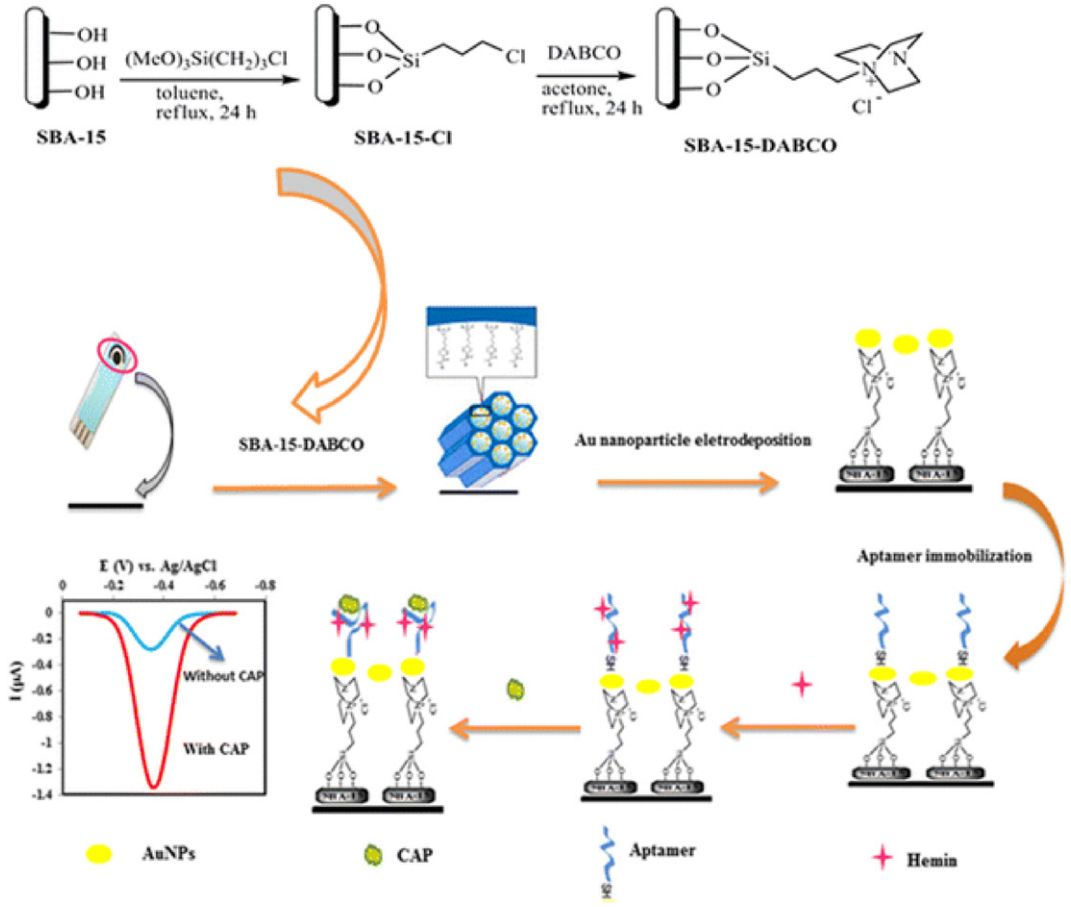

Fig. 4. Scheme of the label-free aptasensor for chloramphenicol detection [44]. A graphite SPE was modified with a dendritic nanostructure based on mesoporous silica (SBA-15) functionalized with 1,4-diazabicyclo [2,2,2]octane (DABCO) in order to increase Au nanoparticles immobilization, enhancing surface conductivity. The specific aptamer was bound via Au—S interaction, and analyte capturing was detected via DPV, using hemin as electrochemical. Reprinted with Springer permission.

to the aptasensor robustness. By relying on EIS of $[Fe(CN)_6]^{3-/4-}$ at the aptasensor as a calibration parameter, a LOD of 14×10^{-9} M ochratoxin-A was obtained. The aptamer-based assay is shown in Fig. 5.

Catanante et al. [73] reported an OTA aptasensor based on a MB conjugated aptamer immobilized on a graphite SPE. Several aptamer immobilization methods including covalent immobilization, diazonium chemistry and adsorption technique were studied. Among them, grafting hexamethylenediamine on the electrode surface as aptamer linker was found to yield the optimum electrochemical signal. Interaction between OTA and the aptamer then yielded a conformational change that positioned MB closer to the working electrode to produce an enhanced signal. The authors estimated a LOD of 2×10^{-11} M OTA based on the DPV detection of MB. Mishra et al. [57] reported an OTA aptasensor developed using two different strategies. The first strategy was based on a label free approach in which the aptamer was covalently immobilized on a graphite SPE surface. The interaction between the aptamer and OTA was then followed using EIS measurements of $[Fe(CN)_6]^{3-/4-}$ at the aptasensor obtaining a LOD of 3.7×10^{-10} M OTA. The second approach was based on competitive approach between un-labeled and biotin-labeled target with a covalently immobilized aptamer [175]. The analytical signal was amplified after binding avidin-alkaline phosphatase to labeled hybridized aptamer. Alkaline phosphatase then catalyzed conversion of 1-naphtyl-phoshpate to 1-naphthol the oxidation of which was expected to be inversely proportional to ochratoxin-A concentration and a LOD of 2×10^{-10} M was achieved. An OTA aptasensor constructed by immobilizing a selected aptamer on graphene oxide modified graphite SPE was reported by Bulbul et al. [46]. This aptasensor functioned based on the competition between un-labeled and nanoceria-labeled ochratoxin-A. The decrease of the oxidation current signal of H_2O_2 catalyzed by nanoceria was measured with cyclic voltammetry when the analyte concentration increased. A LOD of 0.1×10^{-6} M ochratoxin-A was estimated. Sun et al. [39] attached a thionine-labeled aptamer

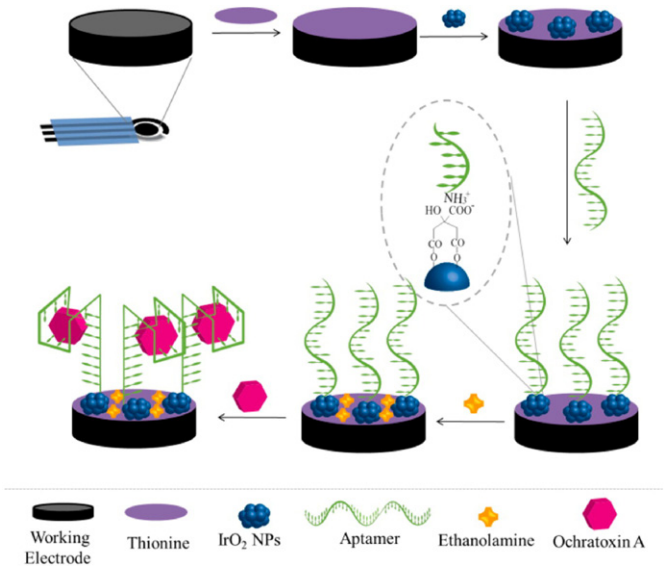

Fig. 5. Scheme of the label-free impedimetric aptasensor for ochratoxin-A detection [48]. A graphite SPE was modified with a thin film of electrodeposited polythionine and IrO_2 nanoparticles, on which the probe aptamer was electrostatically immobilized. The sensor was able to detect the target in buffered samples by means of EIS, using $[Fe(CN)_6]^{3-/4-}$ as redox probe. Reprinted with permission from American Chemical Society.

to graphene nanosheets via non-covalent interaction. The electronic signal was acquired via negatively charged carbon SPE toward free thionine molecules. Initially, the formed thionine–aptamer/graphene nanocomposites were suspended in the detection solution and far away from the electrode, thereby resulting in a weak electronic signal. Upon addition of target OTA, the analyte reacted with the aptamer causing the dissociation of thionine–aptamer from the graphene oxide nanosheets. The newly formed thionine–aptamer/OTA could be readily cleaved by DNase I. Free OTA, was used to trigger again aptamer release, while free thionine was used as electrochemical mediator being measured by negatively charged graphite SPE via DPV. A LOD of 1.4×10^{-11} M was obtained and real-life samples were also tested, using spiked and naturally contaminated wheat.

Istamboulié et al. [84] developed an impedimetric aptasensor for aflatoxin-M1 detection, based on graphite SPE on which a hexaethyleneglycol-tailored aptamer was immobilized via carbodiimide binding. Aptamer-target interaction induced an increase of electron-transfer resistance of $[Fe(CN)_6]^{3-/4-}$ at the electrode measured by EIS. The authors estimated a LOD of 3.5×10^{-12} M aflatoxin-M1.

Yugender Goud et al. [58] reported a label free aptasensor for aflatoxin-B1 detection based on graphite SPEs on which two different probe aptamers (seqA and seqB) were separately linked via covalent bond. Interaction with aflatoxin-B1 caused an increase of electron transfer resistance of $[Fe(CN)_6]^{3-/4}$ at the electrode that was monitored by EIS. LODs of 3.8×10^{-10} M for seqA and 8.0×10^{-10} M for seqB were achieved.

Clostridium difficile is a pathogen often involved in nosocomial intestinal infections and toxin-A is a lethal enterotoxin produced by this organism. Clostridium difficile was also isolated from different environmental sources, such as soil and various water environments (seawater and freshwater) [176–178]. Luo et al. [41] described an aptamer-based biosensor for toxin-A detection using gold nanoparticles synthesized by *Bacillus stearothermophilus*. A thiolated oligonucleotide sequence used as the capture probe was immobilized on the Nafion/thionine/AuNPs-modified SPE via Au—S binding. The horseradish peroxidase (HRP) labeled aptamer probe was then hybridized to the immobilized complementary capture probe to form an aptamer–DNA duplex. In the absence of toxin-A, the aptamer–DNA duplex modified the electrode surface with HRP, so that an amperometric response was induced based on the electrocatalytic properties of thionine. This was mediated by the electrons that were generated in the enzymatic reaction of H_2O_2 under HRP catalysis. In presence of toxin-A, a toxin-A-aptamer complex was produced, forcing the HRP-labeled aptamer to dissociate from the electrode surface, which reduced the catalytic capacity of HRP and thus reduced the response current. The authors evaluated a LOD 1×10^{-6} M toxin-A by amperometric detection of H_2O_2 at the electrode.

2.3.4. Aptasensors for metals

Environmental arsenic contamination can cause a series of health problems, including systemic disease and carcinogenic effects [179–182]. Toxicity and mobility of arsenic depend on its oxidation state. The trivalent As(III) is more mobile and toxic than As(V) and organic arsenic compounds [183], because of its ability to form complexes with certain co-enzymes. Cui et al. [74] described an aptasensor for arsenite ion detection based on graphite SPE on which gold nanoparticles were deposited for aptamer immobilization. The arsenite(III)-aptamer adsorbed cationic polydiallyldimethylammonium (PDDA) via electrostatic interaction to repel other cationic species. In the presence of arsenite, the change of aptamer conformation due to the formation of arsenite-aptamer complex led to less adsorption of PDDA, and the complex adsorbed more positively charged $[Ru(NH_3)_6]^{3+}$ as an electrochemically active indicator on the aptasensor surface, which produced a sensitive turn-on response. Quantitative analysis was based on DPV detection of $[Ru(NH_3)_6]^{3+}$ at the electrode and a LOD of 0.15×10^{-6} M arsenite was achieved.

3. Conclusions

In recent years, there have been many reports describing advances in aptamer developments, as well as combination of aptamer with nanomaterials in order to improve detection procedures. Compared to other techniques, electrochemical transduction has shown advantages including the possibility to integrate different signal amplification schemes (e.g. using catalytic or bio-catalytic amplification) by simply introducing a suitable label to a DNA probe sequence or to the aptamer-target complex. Many assays were developed using a label free approach, in order to simplify step-by-step analytical procedure.

This review has shown that aptasensors are among the most promising approaches for biosensor development. Nonetheless, there is still a lack of applications of aptasensors to environmental analysis, albeit there are many examples in which aptasensors were used to detect clinical targets.

Aptasensors for food safety and environmental analysis are under a developmental phase compared to immunoassays. The primary difficulty is the limited number available oligonucleotide sequences (even if SELEX continuous improvements will help scientists from this point of view). Another potentially growing field concerns development in aptamer immobilization strategies, especially in combination with nanomaterials. These limits certify that exciting improving opportunities still exist in the field of aptasensors. Future growth could be focused on the design of aptamers against unexplored target analyte, and then their subsequent integration in well-known and reliable electrochemical platform to perform analysis.

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