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Electrochemical biosensors based on nanomaterials for aflatoxins detection: A review (2015–2021)

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HIGHLIGHTS

- Aflatoxins (AFs) are important natural contaminants of a wide variety of agricultural products.
- Electrochemical biosensors are ideal devices for AFs *in field* analysis.
- Current trends rely in novel nanomaterials for improving the systems.
- Authoritative opinion on electrochemical biosensors based on nanomaterials for AFs is given.
- 2015–2021 literature, together with future prospects and challenges is discussed.

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ABSTRACT

Due to their immunotoxicity, aflatoxins are considered as very important natural contaminants of a wide variety of agricultural products. Although conventional techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) combined with mass spectrometry (MS) have traditionally been used to detect such food contaminants, they are relatively expensive, laborious, and time consuming which limits their use for *in field* analysis at the so-called point-of-care (POC). Electrochemical biosensors are emerging devices that meet these expectations since they rely in reliable, simple, inexpensive, portable, selective, and easy-to-use analytical procedures and instruments that can be used by unskilled personnel outside the laboratory. In this context, in this review article we summarize and provide authoritative opinion on the use of electrochemical biosensors for aflatoxins of interest for food control. Even though previous reviews have rightly covered this issue, the continuous research and improvements in this field, mostly related to the use of novel nanomaterials, make an update much needed. For this reason, this review covers the most relevant approaches reported in the period 2015–2021, focusing for the first time on the use of nanomaterials for improving the biosensors performance. The principles of the different strategies developed are discussed, and some examples of relevant approaches are highlighted, together with future prospects and challenges.

1. Introduction

Aflatoxins (AFs) are a specific type of mycotoxins produced by various filamentous fungi, which are able to spread in food for consumption. Like all other types of toxins, they are toxic compounds that may cause disease in living things exposed to them. In particular, they are secondary carcinogenic metabolites, classified by the IARC (International Agency for Research on Cancer) as group 1 ones, as they are part of the carcinogens in humans [1,2]. AFs bind to DNA, RNA and proteins forming AF-DNA adducts that accumulate over years and damage viruses in humans [3].

AFs are the most studied mycotoxins, due to their immunotoxicity. They are considered natural contaminants of a wide variety of agricultural products, such as corn. They affect a wide range of unfermented and fermented foods due to their richness in compounds that promote synthesis [1]. They were discovered in the United Kingdom in 1960 after the death of 100,000 turkeys, eaten with peanut food contaminated with Brazilian AFs [1,2,4], and were called aflatoxins due to the combination of a = Aspergillus, fla = flavus and toxins = poison [3], as it was the *Aspergillus flavus* fungus what caused the poisoning. However, later it

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turned out that there are several fungi within the same family that are able to produce these toxins.

There are up to 18 types of AFs, but only 6 of them are regularly regulated for food control since they are the most harmful, with prominent presence in the environment: AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2, being AFB_1 the most dangerous one [4,5]. While the first four are produced by fungi from the *Aspergillus* family, AFM_1 and AFM_2 are generated by some mammals, such as cows, after consuming food contaminated with AFB_1 or AFB_2, respectively. These are undoubtedly much less dangerous to human health than the original AFs. However, their control is important because they are secreted by the cow in the milk, a product associated with children and babies, and are not removed during pasteurization. The structure of all these AFs is shown in Fig. 1.

The Food and Agriculture Organization (FAO) estimates that mycotoxins affect a quarter of crops worldwide, including staple foods such as corn, cotton seeds and nuts. It is also worthy to mention the contamination of animal products such as eggs, meat or milk as a result of animal ingestion of feed contaminated with mycotoxins [6]. AFB_1, in addition to being the most toxic, is the one with the highest levels of pollution [7]. AFs B_1, B_2, G_1 and G_2 are the major pollutants in rice while AFs M_1 and M_2, as already mentioned, are found in milk.

The clinical manifestations of aflatoxicosis (poisoning caused by the consumption of substances or foods contaminated with aflatoxin) may be of acute type, occurring when large doses of AFs are ingested in a relatively short period of time, and of chronic character. Chronic effects coming from the consumption of food contaminated with low levels of AFs for long periods of time are the most common and difficult to identify [8]. Symptoms of acute aflatoxicosis include sickness, abdominal pain, pulmonary edema, fatty infiltrates and necrosis of the liver [7], which can be fatal, in general, due to liver damage [9]. Chronic exposure affects many organs, being the liver the primary target, so AFs are considered as hepatotoxic in humans and animals, causing cirrhosis (severe liver damage) or liver cancer [10]. The IARC (International Agency for Research on Cancer) has continuously evaluated the carcinogenicity of AFs, concluding that AFB_1 is the most potent biological carcinogen known to date.

In addition to carcinogens, AFs are mutagens and teratogens (agents that produce malformations in the embryo or fetus) in the following order: $AFB_1 > AFM_1 > AFG_1 > AFM_2 > AFB_2 > AFG_2$ [3]. Therefore, chronic exposure to AFs not only causes liver problems but also teratogenesis, immunosuppression and mutagenesis.

Therefore, strict control of the content of AFs in food is necessary. In accordance with the Codex Alimentarius Mundi recommendations, it has been established that the maximum levels of total aflatoxins (AFs B_1, B_2, G_1 and G_2) in food must not exceed 10 μg kg^{-1}. In addition, AFB_1 has an individual MRL of 5 μg kg^{-1} in foods for human consumption and 0.1 μg kg^{-1} in products for infants and young children or dietary foods

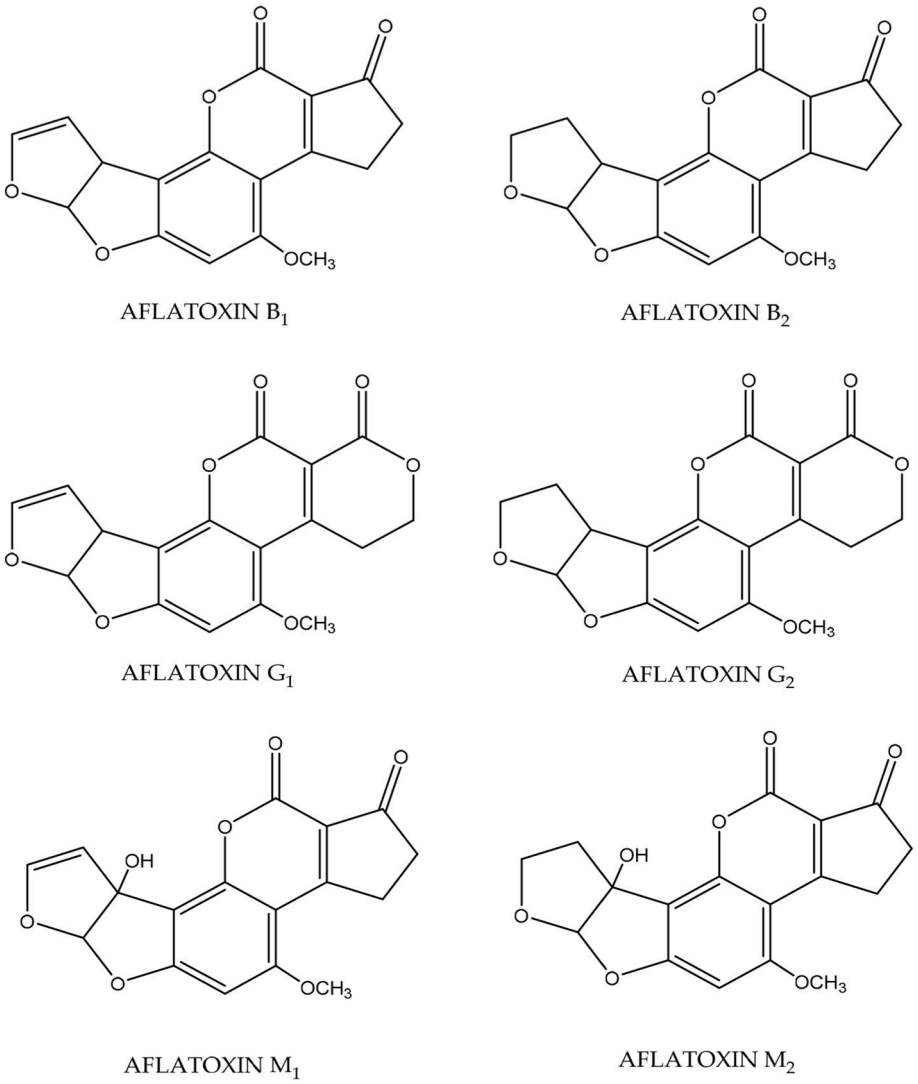


Fig. 1. Chemical structure of the aflatoxins with prominent presence in the environment.

dedicated to special medical use [11]. On the other hand, AFM_1 has a much lower limit, 0.050 μg kg^{-1} for milk and dairy products and 0.025 μg kg^{-1} for those dairy products intended for infant formula.

The conventional analytical methods that meet these specifications are mainly based on thin layer chromatography (TLC), high performance liquid chromatography with fluorescence detection (HPLC-FL), high performance liquid chromatography combined with mass spectrometry (HPLC-MS) and capillary electrophoresis (EC) [12–15]. These methods have high sensitivity and selectivity, but have important limitations related to the requirement of qualified personnel, expensive equipment and large sample volumes, among others.

In this context, biosensors have emerged as outstanding analytical tools for overcoming such limitations [16]. The main biological materials used in biosensing technology are enzyme/substrate, antibody/antigen, and nucleic acid/complementary sequence pairs. The selectivity of the biological detection element offers the possibility to develop highly specific devices for the real-time analysis of complex mixtures [17].

In particular, electrochemical biosensors benefit from the inherent advantages of the electrochemical techniques of analysis, in terms of robustness, excellent detection limits, wide range of response, good stability and reproducibility, low sample volume need and easy miniaturization, as well as ability to be used in turbid media containing compounds with optical and fluorescent properties [18,19]. Moreover, the rapid and simple experimental procedures required allow to use cheap/portable equipment, which is highly suitable for point-of-care (POC) applications [20].

Recent trends in electrochemical biosensors point to the working electrode modification with nanomaterials such as nanoparticles (i.e. Cu, Ni, Au, Ag …), carbon nanotubes (CNTs), carbon nanofibers (CNFs), graphene oxide (GO), reduced graphene oxide (rGO) or quantum dots (QDs), with the aim of improving the biosensor performance [21–25]. Most of the works found in the bibliography make use of glassy carbon electrodes (GCEs), but the use of screen-printed electrodes (SPEs) has shown a high increase in the last years, taking advantage of their great versatility, small size, small sample volume required and low cost, which is ideal for POC analysis [26–29].

The challenges and perspectives of the electrochemical biosensors applied for food safety, including the analysis of toxins, pathogens, pesticides, antibiotics, endocrine-disrupting chemicals, and allergens have been recently reviewed [30]. Regarding mycotoxins analysis, recent reviews have focused both on analytical methods in general [31–39] and on electrochemical approaches in particular [40–45], also including photoelectrochemical ones [46]. Statistics of publications over the years according Web of Science show how that this field is rapidly evolving in the last years: Searching by the terms “aflatoxin”, “electrochemical” and “biosensor”, 198 publications are found (80% in the last 10 years; 70% from 2015) while the search by “mycotoxin” instead of “aflatoxin” gives 148 publications (77% in the last 10 years; 68% from 2015).

Even though such previous reviews have rightly covered this issue, the continuous research and improvements in this field, mostly related to the use of novel nanomaterials, make an update much needed. For this reason, this review covers the most relevant approaches reported in the period 2015–2021, focusing for the first time on the use of nanomaterials for improving the biosensors performance.

The following sections summarize the most representative electrochemical biosensors based on nanomaterials for aflatoxins detection reported in the period 2015–2021, classified according to the bioreceptor used: i) immunosensors, ii) aptasensors and iii) other approaches (direct detection of electroactive aflatoxins, enzymatic sensors and molecularly imprinted polymer (MIP)-based sensors) (Fig. 2). Due to its greater toxicity, most of the reports found in the bibliography so far are focused on the detection of AFB_1, and in a minor extent, of AFM_1 and

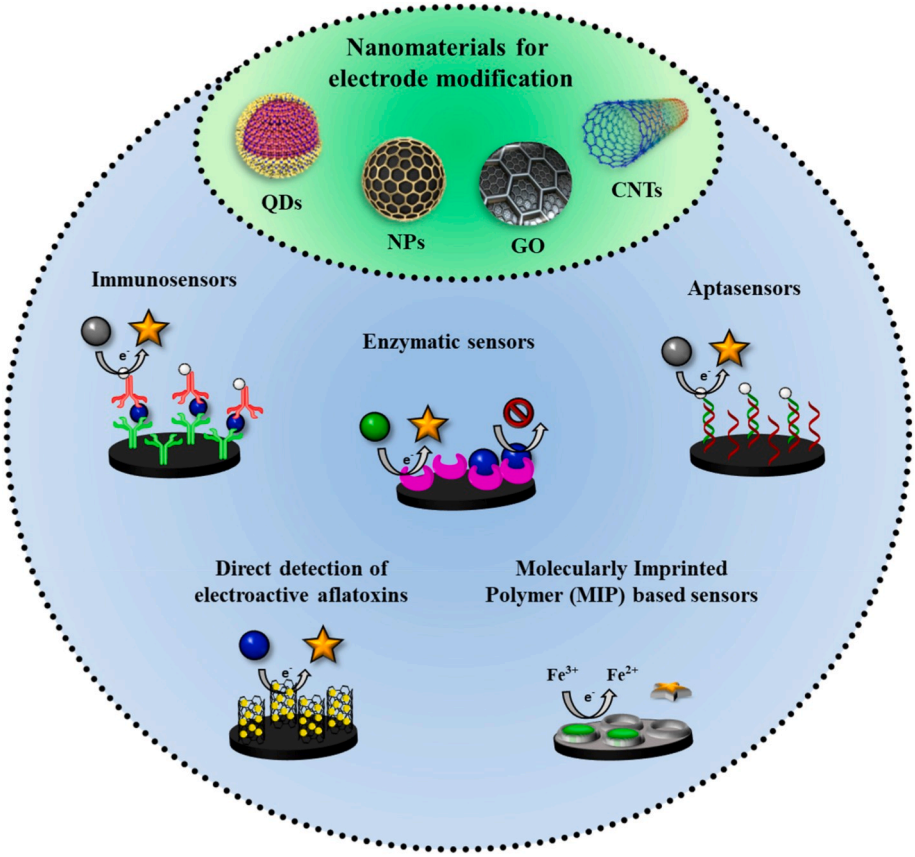


Fig. 2. Main biosensing routes followed for the electrochemical detection of aflatoxins based on nanomaterials.

AFB_2. A critical vision on the advantages, drawbacks and perspectives is also given in this review.

2. Electrochemical immunosensors

As previously mentioned, an immunosensor is a biosensor that uses an antibody (Ab) as a biological recognition element of the antigen (Ag), in this case aflatoxin. Antibodies are proteins generated by living organisms in the presence of foreign substances, antigens, that are recognized and subsequently eliminated by that organism. The main advantages of the immunosensors are related to the high stability, selectivity and specificity of antibodies.

The small size of AFs limits the development of sandwich-based approaches, so the detection strategies are basically based on i) label-free assays, with either (volt)amperometric or electrochemical impedance spectroscopy (EIS)-based detection and ii) competitive assays, using enzymatic and nanoparticle labels and voltammetric or photoelectrochemical detection.

2.1. Label-free immunosensors

The decrease in the electronic transference on the electrode surface upon the immunocomplex formation has been approached for the AFs detection using different assay formats, materials and detection modes. In most cases, electrode surface modification with different nanomaterials has been proposed for both enhancing the electronic transference and also to improve the efficiency of the antibody immobilization, leading to a better performance of such label-free approaches. That is the case of the work reported by Zhao's group where they used a glassy carbon electrode (GCE) modified with 4-aminobenoic acid, reduced graphene oxide and gold nanoparticles (AuNPs-PABA-rGO) for the detection of AFB_1 [47] (Fig. 3A). Such nanocomposite also allows to better immobilize the anti-AFB_1 antibodies in an oriented manner, thanks to the carbodiimide chemistry. EIS-based detection allowed to detect the toxin at levels of ng mL^{-1} even in samples of vegetable oil. Similarly, Wang et al. proposed the use of reduced graphene oxide-pyrrolepropyl acid (rGO-PPa) nanocomposites for the modification of a GCE [48]. rGO improves the electrode conductivity while pyrrolepropyl acid (PPa) provides the functional groups for the covalent immobilization of the antibody. The combination of such materials allows to determine AFB_1, lowing at levels of pg mL^{-1} even in corn samples.

Metallic nanoparticles have also been proposed as alternative to carbon-based nanomaterials for the modification of the electrode surface. As example, the excellent conductivity of gold nanoparticles (AuNPs) together with their affinity to antibodies has been approached for their deposition on indium tin oxide (ITO) and further anti-AFB_1 antibodies immobilization [49] (Fig. 3B). The decrease in the voltammetric signal of the $[Fe(CN)_6]^{3-/4-}$ redox couple upon AFB_1 recognition allows the detection of such toxin in corn samples at pg mL^{-1} levels.

In addition to the above detailed representative approaches, many other works based on the same principles have been reported in the period 2015–2021, as summarized in Table 1. Such label-free approaches have important advantages related to simplicity, speed and cost of analysis. However, and despite the efforts in the integration of nanomaterials for improving the sensitivity of these approaches, the limits of detection reached are typically at levels of ng mL^{-1}, which in some cases is not enough for fulfilling the legislation requirements. Moreover, EIS-based detection often suffers from unspecific signals coming from electrode fouling, what limits its implementation for real sample analysis. In this context, competitive immunosensors have been proposed for overcoming such limitations.

2.2. Competitive immunosensors

Competitive immunoassays using either enzymatic or nanoparticle labels [50] have also been proposed for a more sensitive detection of AFs. As in the label-free approaches, nanomaterials have been extensively used as electrode modifiers for improving the performance of the sensing system.

2.2.1. Voltammetric immunosensors

Screen-printed carbon electrodes (SPCE) have been modified with composites made of multiple-walled carbon nanotubes and chitosan (MWCNTs-Chi), which benefits of the high conductivity of the carbon nanomaterial and the biofunctionality of chitosan for covalently binding a AFB_1 conjugate with bovine serum albumin (BSA-AFB_1) [51] (Fig. 3C). Then the free analyte (AFB_1) and the immobilized on the electrode surface compete for the specific antibody (anti-AFB_1). After incubation with a secondary antibody labelled with HRP, the electrochemical reduction of the enzymatically oxidized TMB substrate is voltammetrically monitored, allowing to detect AFB_1 at levels as low as pg mL^{-1}. Similar approaches, but using single-wall carbon nanotubes (SWCNTs) as electrode modifiers for the immobilization of, in this case, the anti-AFM_1 antibody and using an enzyme-labelled antigen (AFM_1-HRP) have been reported for the detection of such toxin in milk samples [52]. In the same line, Chauhan et al. [53] developed a competitive immunosensor on gold electrodes, using antibodies labelled with core@shell Fe_3O_4@Au nanoparticles and voltammetric detection (Fig. 3D). This design allowed to improve the LOD of AFB_1, reaching the pg mL^{-1} level. Moreover, the magnetic properties of the label allowed to propose this approach as a reusable sensor, thanks to the action of an external magnet that is able to remove the AFB_1-IgG-Au-Fe_3O_4 conjugate.

In addition to enzymatic labels, metallic nanoparticles have also shown excellent properties for their use as tags in competitive immunoassays for AFs detection. In particular, it is worthy to be highlighted the recently reported combination of platinum nanoparticles with reduced graphene oxide (Pt-rGO) for their use as antibody tags for AFB_1 detection [54]. BSA-AFB_1 conjugates are immobilized in a AuNP-modified GCE. After competition between the AFB_1 in the electrode and the free analyte for the Pt-rGO-labelled antibody, the analytical signal is recorded through the electrochemical reduction of H_2O_2 by the Pt tags. In this case, pg mL^{-1} levels of toxin are detected, also benefiting of the higher stability of the metallic label and the simplicity of the measurement, compared with the more tedious enzymatic assay.

2.2.2. Photoelectrochemical immunosensors

Photoelectrochemical (PEC) immunosensors based on nanomaterials have also been recently applied for aflatoxins detection. Such approaches consist in the use of light as excitation source and photocurrent as analytical signal, benefitting from lower background noise and higher sensitivity compared to voltammetric techniques [55,56]. Different nanomaterials have been proposed as photoactive material modifying the working electrode to generate photocurrent signal under light irradiation.

In the most representative recent approaches, fluorine-doped tin oxide (FTO) electrodes are modified with materials such as $CsPbBr_3$/TiO_2 nanocrystals [57], CdS nanoparticles covered by cobalt oxyhydroxide (CoOOH) nanosheets [58], Mn^{2+}-doped $Zn_3(OH)_2V_2O_7{\cdot}2H_2O$ nanobelts [59], carbon quantum dots (CQDs)-functionalized MnO_2 nanosheets [60] and cerium-doped Bi_2MoO_6 (Ce-Bi_2MoO_6) nanosheets [61] for the development of competitive immunoassays for AFB_1 detection, reaching limits of detection at levels of pg mL^{-1}. It is also worthy to mention the use of Quartz Crystal Microbalance (QCM) platforms combined with nanoliposome carriers for signal amplification purposes [62], as well as CdTe QDs-modified glassy carbon electrodes (GCE) in combination with silver nanoparticle (AgNPs) tags for assisting an ion-exchange reaction responsible of the photocurrent generation [63]. In both cases AFB_1 is also detected at pg mL^{-1} levels.

Some other works based on similar principles have been reported in the last years (Table 1). As general remark, competitive assays allow to

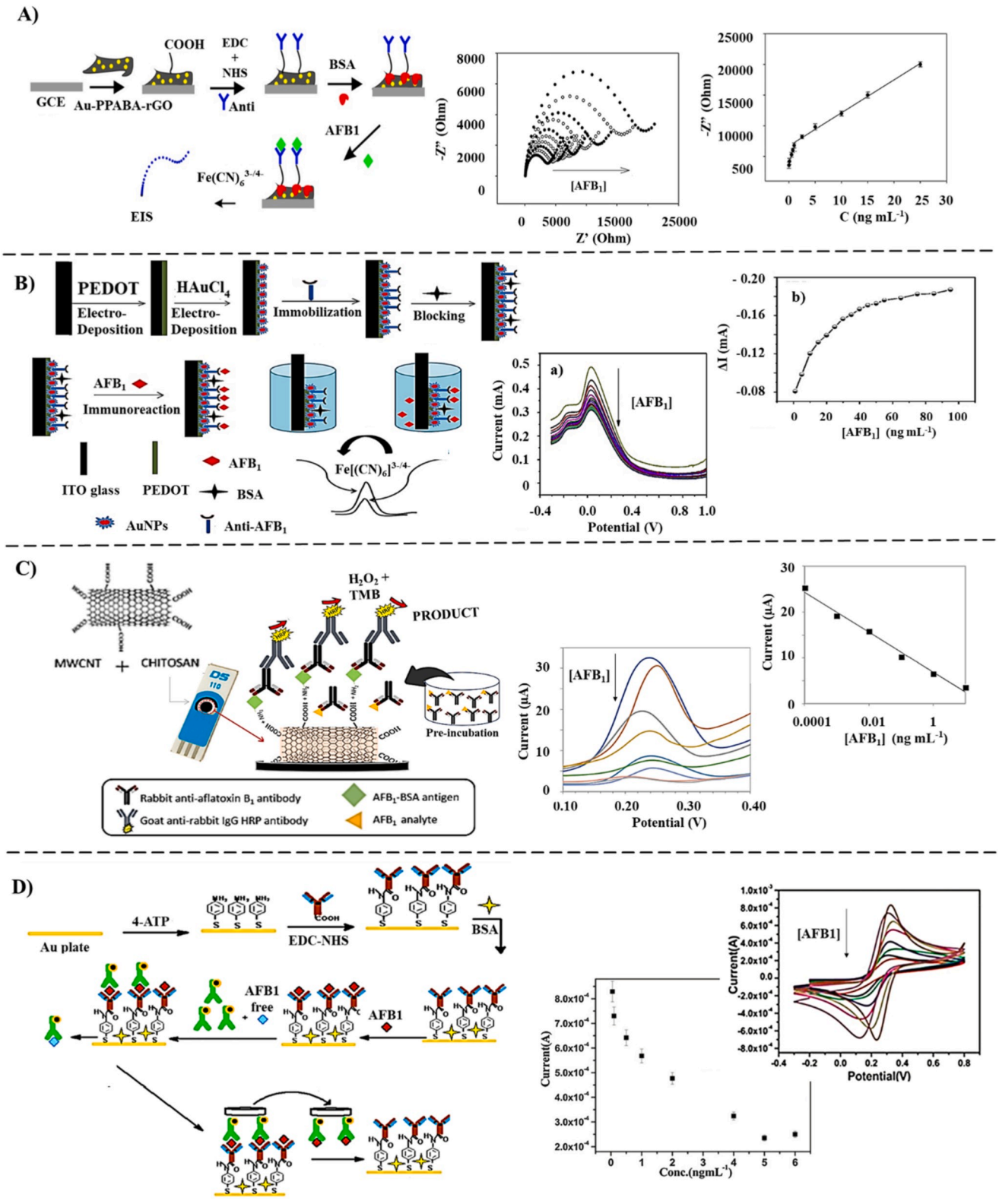


Fig. 3. Electrochemical immunosensors based on nanomaterials for aflatoxins detection. A) Label-free immunosensor for AFB_1 detection on GCE electrodes modified with AuNPs-PABA-rGO. EIS spectrum for different AFB_1 concentrations and calibration curve. Adapted from Ref. [47] with permission. B) Label-free immunosensor for AFB_1 detection on ITO electrodes modified with AuNPs. DPVs for different concentrations of AFB_1 (a), calibration plot (b) and linear ranges (c–d). Adapted from Ref. [49] with permission. C) Competitive immunosensor for AFB_1 detection on SPCE electrodes modified with MWCNTs-Chi, using HRP tags. DPVs for different concentrations of AFB_1 and calibration curve. Adapted from Ref. [51] with permission. D) Competitive immunosensor for AFB_1 detection on gold electrodes using antibodies labelled with core@shell Fe_3O_4@Au nanoparticles. Calibration curve of the immunosensor at different concentrations of AFB_1. Adapted from Ref. [53] with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Electrochemical immunosensors based on nanomaterials for aflatoxins detection reported in the period 2015–2021.

Immunoassay	Nanomaterial for electrode modification	Technique*	Analyte	Lineal range	LOD	Spiked sample/ Recovery	Ref
Label-free	Ab-AFB_1/BSA/AuNPs-PABA/rGO/GCE	EIS	AFB_1	0.01–1 ng mL^{-1} 1–25 ng mL^{-1}	0.001 ng mL^{-1}	Vegetable oil/Unspiked	[47]
	Ab-AFB_1/PPy/PPa/rGO/GCE	EIS	AFB_1	0.01–10 pg mL^{-1}	0.01 pg mL^{-1}	Corn/80–112%	[48]
	BSA/Ab/AuNPs/PEDOT/ITO	DPV	AFB_1	1–25 ng mL^{-1}	4.5 pg mL^{-1}	Maize/96-94%	[49]
	Ab/AFB_1-BSA/Au colloid/GE	EIS	AFB_1	0.08–100 ng mL^{-1}	0.05 ng mL^{-1}	Rice/88–104%	[64]
	Ab/MWCNTs/RTIL/GCE	EIS	AFB_1	0.1–10 ng mL^{-1}	0.03 ng mL^{-1}	Olive oil/96–116%	[65]
	Ab/BSA-EDC-NHS/SPGE	EIS	AFB_1	1–20 ng mL^{-1}	0.5 ng mL^{-1}	Plant extracts/96–104%	[66]
	Ab-AFB_1/Cys-MWCNTs/GE	EIS	AFB_1	0.1–20 pg mL^{-1}	0.79 pg mL^{-1}	Corn flour/Unspiked	[67]
	Ab-AFB_1/BSA/AuNPs-COOH-GO/GCE	EIS	AFB_1	0.05–25 ng mL^{-1}	0.05 ng mL^{-1}	Rice/99–101%	[68]
	BSA/Ab-AFB_1/Chi-AuNPs/GME	DPV	AFB_1	0.1–1 ng mL^{-1} 1–30 ng mL^{-1}	0.06 ng mL^{-1}	Maize/97–110%	[69]
	Ab/MWCNTs/PDDA/Pd-AuNPs/GE	DPV	AFB_1	0.05–25 ng mL^{-1}	0.03 ng mL^{-1}	Rice/98–103%	[70]
	Ab/AuNPs/PANI/GN/SPGE	DPV	AFB_1	0.05–25 ng mL^{-1}	0.034 ng mL^{-1}	Rice/97–104%	[71]
	BSA/Ab/AuNPs/PEDOT-GO/GCE	DPV	AFB_1	0.5–20 ng mL^{-1} 20–60 ng mL^{-1}	0.109 ng mL^{-1}	Corn/92–95%	[72]
	BSA/Ab-AFB_1/Chi-AuNPs/GME	CV	AFB_1	0.2–2 ng mL^{-1} 2–30 ng mL^{-1}	0.12 ng mL^{-1}	Wheat/90–106%	[73]
	BSA/Ab-AFB_1/nBi_2O_3/ITO	CV	AFB_1	0.01–0.7 ng mL^{-1}	0.0872 ng mL^{-1}	–	[74]
	Ab-AFB_1/AuNPs/rGO/ITO	CV	AFB_1	0.001–100 ng mL^{-1}	6.9 pg mL^{-1}	Peanut/98–103%	[75]
	SA/Biotin Ab-AFB_1/SPE	CV	AFB_1	0.00005–5 ng mL^{-1}	50 fg mL^{-1}	Rice milk/Unspiked	[76]
	BSA/Ab-AFB_1/Mn_2O_3/ITO	DPV	AFB_1	1 pg mL^{-1} – 10 μg mL^{-1}	0.54 pg mL^{-1}	Sweet corn/96–99%	[77]
Competitive	Ab-AFB_1/MWCNTs/Chi/SPCE	DPV (Indirect HRP)	AFB_1	0.0001–10 ng mL^{-1}	0.3 pg mL^{-1}	Peanut/Unspiked	[51]
	Ab-AFM_1/SWCNTs/PET	CA (Direct HRP)	AFM_1	0.01–1 ng mL^{-1}	0.02 ng mL^{-1}	Milk/Unspiked	[52]
	AFB_1/BSA/IgG-Au-Fe_3O_4/Ab-AFB_1/4-ATP/ EQCM	EQCM	AFB_1	0.05–5 ng mL^{-1}	0.07 ng mL^{-1}	Corn flakes/Unspiked	[53]
	Ab-AFB_1/PtNPs/CoTPP/rGO/AFB_1-BSA/ AuNPs/GCE	DPV	AFB_1	0.005–5.0 ng mL^{-1}	1.5 pg mL^{-1}	Peanut/Unspiked	[54]
Competitive	$CsPbBr_3$/TiO_2/FTO	PEC	AFB_1	0.01–15 ng mL^{-1}	2.8 pg mL^{-1}	Peanut/90–104% Corn/95–109%	[57]
	CdS/CoOOH/FTO	PEC	AFB_1	0.01–10 ng mL^{-1}	2.6 pg mL^{-1}	Peanut/Unspiked	[58]
	Mn^{2+}-$Zn_3(OH)_2V_2O_7{\cdot}2H_2O$/FTO	PEC	AFB_1	0.0005–10 ng mL^{-1}	0.3 pg mL^{-1}	Peanut/Unspiked	[59]
	CQDs-MnO_2/FTO	PEC	AFB_1	0.01–20 ng mL^{-1}	2.1 pg mL^{-1}	Peanut/Unspiked	[60]
	Ce-Bi_2MoO_6/FTO	PEC	AFB_1	0.0003–10 ngmL^{-1}	0.1 pg mL^{-1}	Peanut/Unspiked	[61]
	QCM (no electrode modification)	PEC	AFB_1	1.0–10 pg mL^{-1}	0.83 pg mL^{-1}	Peanut/Unspiked	[62]
	CdTe QDs/GCE	PEC	AFB_1	0.01–15 ng mL^{-1}	3.0 pg mL^{-1}	Peanut/Unspiked	[63]
	pAb-AFB_1-MSC-Thi/Nf-GCE	DPV	AFB_1	0.01–20 ng mL^{-1}	3.0 pg mL^{-1}	Peanut/Unspiked	[78]
	AFB_1-BSA-SWCNTs/Chi/GCE	DPV	AFB_1	0.01–100 ng mL^{-1}	3.5 pg mL^{-1}	Corn powder/Unspiked	[79]
	Ab-AFB_1/Fc/MWCNTs/Chi/SPCE	DPV	AFB_1	0.001–20000 ng mL^{-1}	0.159 pg mL^{-1}	Rice/94% Peanut/102% Corn/96%	[80]
	AFB_1-BSA-MWCNTs/Chi/SPCE	DPV (Indirect HRP)	AFB_1	0.0001–10 ng mL^{-1}	0.1 pg mL^{-1}	Feed samples/78–103% Palm kernel cake/ 77–117%	[81]
	BSA-AFM_1/Ab-AFM_1/Ab-ALP/SPGE	DPV (Indirect ALP)	AFM_1	0.01–1000 ng mL^{-1}	0.015 ng mL^{-1}	Milk/Unspiked	[82]
	Ab-AFB_1/AuNPs/AFB_1-BSA-Cu apatite/SPCE	SWSV	AFB_1	0.001–100 ng mL^{-1}	0.2 pg mL^{-1}	Milk/95–110% Peanut oil/90–102%	[83]

* EQCM: Electrochemical Quartz Crystal Microbalance; EIS: Electrochemical Impedance Spectrometry; DPV: Differential Pulse Voltammetry; CV: Cyclic Voltammetry; SWSV: Square Wave Stripping Voltammetry; CA: Chronoamperometry; PEC: Photoelectrochemistry.
Ab: Antibody; BSA: bovine serum albumin; AuNPs: gold nanoparticles; PABA: poly 4-aminobenzoic acid; rGO: reduced graphene oxide; GCE: glassy carbon electrode; PPy: polypyrrole; PPa: pyrrolepropylic acid; PEDOT: poly (3,4-ethylenedioxythiophene); ITO: indium tin oxide electrode; GE: gold electrode; MWCNTs: Multi-walled carbon nanotube; RTIL: ionic liquid at room temperature; EDC: N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride; NHS: N-hydroxysuccinimide; SPGE: screen-printed gold electrode; Cys: cysteine; Chi: chitosan; GME: gold microelectrode; PDDA: poly (diallyldimethylammonium); PANI: polyaniline; GN: graphene; nBi_2O_3: bismuth oxide nanorods; SA: streptavidin; SPE: screen-printed electrode; SPCE: screen-printed carbon electrode; HRP: horseradish peroxidase; PET: polyethylene terephthalate; IgG: immunoglobulin antibody; 4-ATP: 4-aminothiophenol; PtNPs: platinum nanoparticles; CoTPP: 5,10,15,20-tetraphenyl-21H,23H-porphine cobalt; $CsPbBr_3$/TiO_2: amorphous TiO_2 with all-inorganic perovskite $CsPbBr_3$ nanocrystals; FTO: fluorine-doped tin oxide electrode; CdS/CoOOH: CdS nanoparticles covered by cobalt oxyhydroxide nanosheets; Mn^{2+}-$Zn_3(OH)_2V_2O_7{\cdot}2H_2O$: Mn^{2+}-doped $Zn_3(OH)_2V_2O_7{\cdot}2H_2O$ nanobelts; CQDs-MnO_2: carbon quantum dots-functionalized MnO_2 nanosheets; QCM: Quartz Crystal Microbalance; Ce-Bi_2MoO_6/FTO: cerium-doped Bi_2MoO_6 nanosheets; pAb: polyclonal antibody; MSC: mesoporous carbon nanoparticles; Thi: thionine acetate salt; Nf: Nafion; SWCNTs: Single-walled carbon nanotube; Fc: ferrocene; ALP: alkaline phosphatase.

improve the sensitivity of the label-free approaches, typically reaching limits of detection at pg mL^{-1} levels, which in most cases is enough for meeting the legal requirements. This is of special relevance when extraction methodologies involving sample dilution are needed. In contrast, competitive-based methodologies require higher reagents consumption and are more tedious/time consuming. Most of the approaches are applied for the analysis of spiked samples (i.e. corn, maize, rice, wheat, milk, olive oil) with good recovery rates. Only few approaches address the analysis of unspiked samples, where the above mentioned extraction-related issues still represent a limitation.

Overall, the relative high cost and low availability of monoclonal antibodies still remains an important limitation for the immunosensors implementation for AFs analysis. This limitation can be overcame by using aptamers instead of antibodies, as explained in the following section.

3. Electrochemical aptasensors

Aptamers are short sequences (20–90 oligonucleotides) of single-stranded nucleic acids (DNA or RNA) with a specific three-dimensional structure that allows them to bind with high affinity to the target molecule. Etymologically, the term aptamer comes from the Latin *aptus* meaning "to fix" or "to unite" and from the Greek *meros* meaning "particle" [84].

Aptasensors use the aptamer's affinity for the target molecule, in the same way than an immunosensor uses antibodies. Although the use of antibodies has been dominant for the detection of mycotoxins, in recent years aptamers have been proposed as an advantageous alternative due to their inherent benefits: i) immunization of animals is not necessary; they are obtained by in vitro synthesis; ii) lower production cost; iii) better chemical and thermal stability; iv) higher reproducibility; v) smaller size: greater capacity for tissue penetration and adhesion to target molecules; vi) possibility of changing its structure; vii) low toxicity and immunogenicity and viii) not sensitive to denaturation in the presence of solvents frequently used in the extraction of mycotoxins.

Despite the above detailed advantages, the number of aptamers discovered to date for the detection of AFs is still limited. Four are the aptamers specifically developed so far for AFB_1: 5′- GTT GGG CAC GTG TTG TCT CTC TGT GTC TCG TGC CCT TCG CTA GGC CCA CA -3'; 5′-TGG GGT TTT GGT GGC GGG TGG TGT ACG GGC GAG GG-3'; 5′- GAT CGG GTC TGG GTG GCG TAA AGG GAG CAT CGG ACA-3'; 5′- GCA CGT GTT GTC TCT CTG TGT CTC GTG C-3'. A specific aptamer has also been developed for AFM_1: 5′-ACT GCT AGA GAT TTT CCA CAT-3'.

All the studies found in the last 5 years' literature correspond to sensors developed for the detection of AFB_1 or AFM_1, benefitting of the above detailed aptamer sequences, using in most cases electrodes modified with nanomaterials. As in the case of the immunosensors, the main strategies for the detection of AFs using aptamer receptors are mostly based on i) label-free assays, with electrochemical impedance spectroscopy (EIS)-based detection, ii) competitive assays, using enzymatic and nanoparticle labels and voltammetric detection and iii) assays based on conformational changes of the aptamer structure.

3.1. Label-free aptasensors

The simplest strategy for the detection of AFs using aptamer receptors consists in taking advantage of the decrease in the electronic transference on the electrode surface upon the aptamer-AF complex formation. With the aim of pre-concentrating the sample and minimizing matrix effects, recent approaches have also proposed the use of core@shell Fe_3O_4@Au magnetic bead platforms for the immobilization of a thiolated aptamer specific to AFB_1 [85] (Fig. 4A). Increasing concentrations of AFB_1 lead to an increase in the EIS signal measured on SPCEs through the $[Fe(CN)_6]^{3-/4-}$ system. With this approach, AFB_1 is detected in peanut samples at levels of pg mL^{-1}.

Alternatively, nanocomposites such as those made of poly(p-phenylenediamine) and silicon nanoparticles (PpPD-SiNP) have been proposed for the modification of SPCEs, with the aim of amplifying the background capacitive signal [86]. In this particular work, a film of ferrocene (Fc) was then deposited on the nanocomposite-modified electrode. The Fc film has a dual activity here: it serves for the anti-AFM_1 aptamer immobilization and also as redox probe. The changes in the EIS signal coming from the Fc film upon the analyte recognition allows to detect AFM_1 at levels of fg mL^{-1}, without the need of subsequent adding the redox probe.

Many other similar label-free approaches have been reported in the last years, as summarized in Table 2. As in the case of immunosensors, these approaches benefit from the simplicity, speed and cost of analysis, but the limits of detection, typically at levels of ng mL^{-1}, are a limitation in some real sample analyses. As also stated before, the unspecific signals coming from the EIS-based also limit its implementation for real sample analysis. With the aim of overcoming such limitations, competitive aptasensors have also been proposed, as detailed in the following section.

3.2. Competitive aptasensors

The versatility of the aptamers allows to design competitive assays in which DNA sequences complementary to the aptamer (cDNA) are used as effective tools. This is the case of the work developed by Hui et al. for the determination of AFB_1 in peanut and corn samples [87] (Fig. 4B). AuNPs-modified GCEs are used for the immobilization of the thiolated cDNA strand. AFB_1 and cDNA compete for the anti-AFB_1 aptamer, being the DNA duplex formation of lower magnitude as the concentration of AFB_1 increases. A HRP-labelled DNA strand complementary to the cDNA is then used as reporter, being the extent of the DNA hybridization higher when higher is the amount of free cDNA on the electrode. This results in an increased voltammetric signal (catalytic oxidation of hydroquinone (HQ) with hydrogen peroxide (H_2O_2)) for increasing concentrations of AFB_1 in the sample. This allows to detect such toxin in corn and peanut samples, at levels of pg mL^{-1}. A quite similar strategy has been proposed but using chitosan as electrode modifier for the immobilization of the cDNA on SPCEs and a AuNPs-labelled DNA strand complementary to cDNA [88] (Fig. 4C). Interestingly, in this case the competition between the AFB_1 and the cDNA for the aptamer is assisted by magnetic separation, since the thiolated aptamer is previously immobilized on core@shell Fe_3O_4@Au magnetic beads. Finally, the voltammetric signal comes from the electrocatalytic reduction of silver on the AuNPs, detecting AFB_1 at the pg mL^{-1} level.

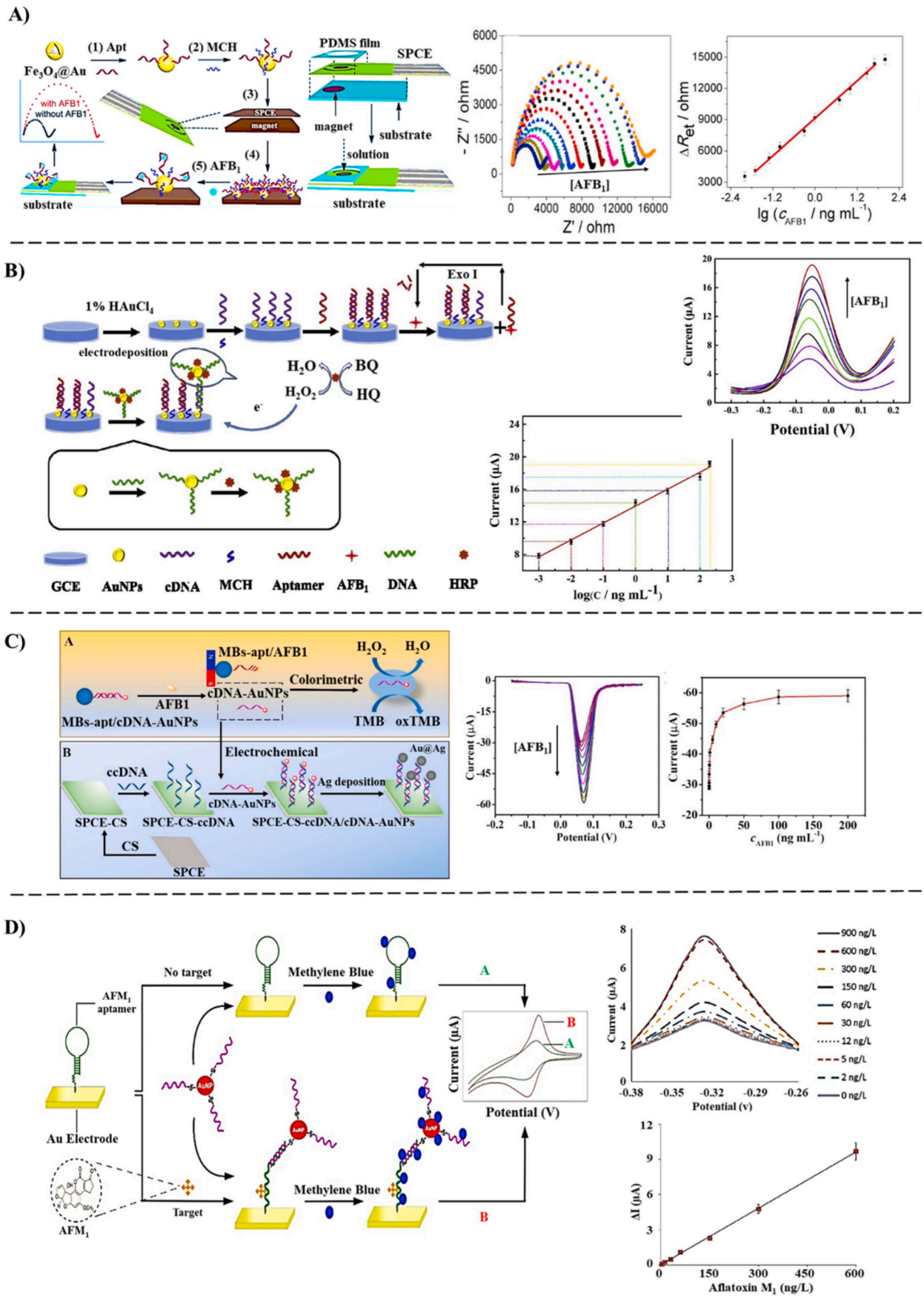
It is also worthy to highlight a similar competitive format using a nanocomposite of thionine and reduced graphene oxide (THI-rGO) for the modification of a GCE in which AuNPs are then deposited has been recently proposed by Li et al. [89]. Thiolated cDNA immobilized on the AuNPs compete with AFB_1 for a ferrocene (Fc)-labelled aptamer. Interestingly, the electroactive THI layer offers a dual detection route: increasing AFB_1 concentrations lead to a decrease in the Fc signal but an increase in the THI one, due to the decrease in the electronic resistance on the electrode. By selecting as analytical signal the ratio of the ones given by both electroactive compounds (I_{THI}/I_{Fc}), a better stability and reproducibility is obtained.

In most cases, the improvement in sensitivity reached by the competitive aptasensors is not so relevant as in the case of the immunosensors, so in general it does not worth to choose this format assay. Actually, few more examples of competitive aptasensors are found in the recent literature, as summarized in Table 2.

3.3. Aptasensors based on aptamer beacons and changes of the aptamer conformation

Aptamer beacons have also been used for AFs detection. Such aptamer structures have a loop region and a terminal stem that forms a closed, hairpin structure. The loop region contains the AFs recognition domain, so in the absence of AF the molecular beacon self-hybridizes at the stem. AF recognition leads to the opening of the loop and the elongation of the aptamer structure, allowing AF detection. Molecular beacon aptasensors are amenable to full regeneration by simply unfolding the aptamer in acidic media (i.e. 1 mol L^{-1} HCl), being this an outstanding advantage compared with other biosensing formats. That is the case of the work recently reported by Taghdisi's group, where a thiolated aptamer anti-AFM_1 is immobilized on a gold electrode and methylene blue (MB) intercalator is used are electrochemical reporter [90] (Fig. 4D). The cDNA is able to hybridize with the aptamer when its hairpin structure is elongated upon AFM_1 recognition. In presence of AFM_1, a higher amount of MB intercalator is captured by the cDNA specifically linked to the electrode, what is enhanced using AuNPs as cDNA carrier. This allows to detect such toxin in milk at pg mL^{-1} levels.

As in the case of the competitive aptasensors, those based on conformational changes of the aptamer structure reported to date do not



(caption on next page)

Fig. 4. Electrochemical aptasensors based on nanomaterials for aflatoxins detection. A) Label-free aptasensor for AFB_1 detection on SPCE electrodes using Fe_3O_4@Au magnetic bead platforms. EIS responses of the aptasensor and calibration plot. Adapted from Ref. [85] with permission. B) Competitive aptasensor for AFB_1 detection on AuNP-modified SPCE electrodes using HRP tags. DPV responses of the aptasensor and calibration plot. Adapted from Ref. [87] with permission. C) Competitive aptasensor for AFB_1 detection on SPCE electrodes using AuNPs tags and further silver deposition. LSV responses and calibration plot. Adapted from Ref. [88] with permission. D) Aptasensor based on changes of the aptamer conformation for AFM_1 detection on SPGE electrodes. DPV responses for methylene blue at different concentrations of AFM_1 and calibration plot. Adapted from Ref. [90] with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Electrochemical aptasensors based on nanomaterials for aflatoxins detection reported in the period 2015–2021.

Aptasensor	Nanomaterial for electrode modification	Technique*	Analyte	Lineal range	LOD	Spiked sample/ Recovery	Ref
Label-free	Apt-Fe_3O_4@Au/SPCE	EIS	AFB_1	0.02–50 ng mL^{-1}	0.015 ng mL^{-1}	Peanut/96–108%	[85]
	Apt/Fc/SiNPs/PpPD/SPCE	EIS	AFM_1	3.3–165 fg mL^{-1}	1.48 fg mL^{-1}	Milk/Unspiked	[86]
	Apt/GA/Cys-PAMAM-G4/GE	EIS	AFB_1	0.03–3 ng mL^{-1}	0.12 ng mL^{-1}	Peanut/91–120%	[91]
	Apt/4-CP/SPCE	EIS	AFM_1	2–150 pg mL^{-1}	1.15 pg mL^{-1}	Milk/99–111%	[92]
	Apt/4-CP/SPCE	EIS	AFB_1	0.125–16 ng mL^{-1}	0.12 ng mL^{-1}	Beer/96–102% Wine/92–101%	[93]
	BSA/Apt/PtNPs/MIL-101(Fe)/GCE	EIS	AFM_1	0.01–80.0 ng mL^{-1}	0.002 ng mL^{-1}	Milk/93–108%	[94]
	MCH/MCH-Apt/AuNPs/BDD	EIS	AFB_1	0.03–3122.8 pg mL^{-1}	0.017 pg mL^{-1}	Peanut/96–109%	[95]
	Apt/BSA/PS-COOH/PDDA-GN/GCE	EIS	AFB_1	0.001–0.1 ng mL^{-1}	0.002 ng mL^{-1}	Oil/98–103% Soy sauce/95–102%	[96]
	Apt/CS1-CS2/SPGE	DPV	AFB_1	7–500 pg mL^{-1}	2 pg mL^{-1}	Human serum/ 95–108%	[97]
	Apt/Cys/GA/PAMAM-G4/GE Biotin-Apt/NA-Fc/GE	DPV	AFM_1	15–120 pg mL^{-1}	8.47 pg mL^{-1} 8.62 pg mL^{-1}	Milk/78–106%	[98]
	Apt/GO/GCE	DPV	AFB_1	0.16–1250 ng mL^{-1}	0.02 ng mL^{-1}	Human plasma/ 98–104% Cow milk/95–103%	[99]
	Apt/AuNRs/GCE	DPV	AFB_1	0.31–78.07 pg mL^{-1}	0.09 pg mL^{-1}	Rice/97–98% Serum/95–107%	[100]
	Apt/GO/PAA/GFE	CA	AFB_1	1–20 ng mL^{-1}	0.13 ng mL^{-1}	–	[101]
	Apt/Cu_2O-CQDs/GCE	EIS	AFB_1	3 ag mL^{-1} −1.9 μg mL^{-1}	0.9 ag mL^{-1}	Wheat flour/97–103%	[102]
	Apt/THI-rGO/GCE	EIS	AFB_1	0.01–100 ng mL^{-1}	0.01 ng mL^{-1}	Peanut/94–116%	[103]
	Apt/THX/GCE	EIS	AFM_1	20–200 pg mL^{-1}	5 pg mL^{-1}	Milk/80–85%	[104]
Competitive	DNA-AuNPs-HRP/Apt/cDNA/AuNPs/GCE	DPV (HRP)	AFB_1	0.001–200 ng mL^{-1}	0.33 pg mL^{-1}	Peanut/94–107% Corn/86–110%	[87]
	cDNA-AuNPs/ccDNA-Chi/SPCE	LSV (Au@Ag)	AFB_1	0.05–100 ng mL^{-1}	0.43 pg mL^{-1}	Corn/93–98%	[88]
	Fc-Apt/cDNA/AuNPs/THI-rGO/GCE	ACV	AFB_1	0.05–20 ng mL^{-1}	0.016 ng mL^{-1}	Peanut/87–112%	[89]
	SA-ALP/Biotin-Apt/AFB_1-BSA/AuNPs-PANI/ GSPE	DPV (ALP)	AFB_1	0.1–10 ng mL^{-1}	0.086 ng mL^{-1}	Corn/80–88% Maize flour/84–96%	[105]
Aptamer beacons	MB-Apt/CS-AuNPs/SPGE	DPV (MB)	AFM_1	2–600 pg mL^{-1}	0.9 pg mL^{-1}	Milk/91–97%	[90]
	MB-Apt/HDMA/FGO/SPCE	DPV (MB)	AFB_1	0.05–6.0 ng mL^{-1}	0.05 ng mL^{-1}	Beer/84–88% Wine/86–89%	[106]
	MB-Apt/HDMA/PT3C/SPCE	DPV (MB)	AFB_1	2.5–30 pg mL^{-1}	1.6 pg mL^{-1}	Coffee/88–93%	[107]
	MB-Apt/cDNA/GE	SWV (MB)	AFB_1	0.6–1250 ng mL^{-1}	0.6 ng mL^{-1}	Beer/97–117%	[108]

* EIS: Electrochemical Impedance Spectrometry; DPV: Differential Pulse Voltammetry; CA: Chronoamperometry; LSV: Linear Sweep Voltammetry; ACV: Alternating Current Voltammetry SWSV: Square Wave Stripping Voltammetry.
Apt: aptamer; Fe_3O_4@Au: core/shell magnetic beads; SPCE: screen-printed carbon electrode; Fc: ferrocene carboxylic acid; SiNPs: silicon nanoparticles; PpPD: poly(p-phenylenediamine); GA: glutaraldehyde; Cys: cystamine; PAMAM: polyamidoamine dendrimers; G4: fourth generation; GE: gold electrode; 4-CP: 4-carboxyphenyl diazonium; BSA: bovine serum albumin; PtNPs: platinum nanoparticles; MIL-101 (Fe): MOF (metal-organic frameworks) with iron as the central metal; GCE: glassy carbon electrode; MCH: 6-mercapto-1-hexanol; AuNPs: gold nanoparticles; BDD: boron doped diamond electrode; PS-COOH: carboxyl-functionalized polystyrene nanospheres; PDDA: poly (diallyl dimethylammonium); GN: N-doped graphene nanosheets; CS1: complementary strand1; CS2: complementary strand2; SPGE: screen-printed gold electrode; Cys: cystamine; NA: neutravidin; GO: graphene oxide; AuNRs: gold nanorods; PAA: polyacrylic acid; GFE: gold film electrode; Cu_2O-CQDs: Carbon quantum dots/octahedral Cu_2O nanocomposite; THI: thionine; rGO: reduced graphene oxide; THX: thiacalix [4]arene nanoparticles; DNA: deoxyribonucleic acid; HRP: horseradish peroxidase; cDNA: complementary strand of DNA; ccDNA: complementary strand of cDNA; Chi: chitosan; SA-ALP: streptavidin-alkaline phosphatase; PANI: polyaniline; GSPE: graphite screen-printed electrode; MB: methylene blue; CS: complementary strand of aptamer; HDMA: hexamethylenediamine; FGO: functional graphene oxide; PT3C: polythiophene-3-carboxylic acid.

allow to significantly improve the sensitivity of label-free approaches. Moreover, the selection of the suitable aptamer structure significantly complicates the design of the assay. This is probably why few more similar works following this detection principle are found in the recent bibliography, as shown in Table 2.

As in the case of the immunosensors, unspiked samples hardly been analysed with aptasensors, being almost of the approaches applied for the analysis of spiked ones, reaching good recovery rates.

4. Other electrochemical approaches

In addition to the widely reported immunosensors and aptasensors, alternative electrochemical approaches for AFs detection are found in the recent bibliography, as detailed in the following sections.

4.1. Direct detection through AFs electroactive groups

AFs are electroactive compounds, since they contain an alcohol group (generated by the hydrolysis of the aromatic ester group in basic medium) that can be electrochemically oxidized to ketone. This allows their direct detection without the need for recognition elements or tags. However, the low specificity of this type if detection, together with its low sensitivity, makes very limited its implementation for the determination of AFs. Actually, only the work published in 2020 by Gevaerd et al. [109] for the determination or AFB_1 has been found in last 5 years' bibliography (Fig. 5A). To do this, they modified the surface of the SPCE electrode with a nanocomposite made of gold nanoparticles and graphene quantum dots (AuNPs-GQDs). They observed that such nanocomposite exerted an electrocatalytic effect on the oxidation of AFB_1 (shift of the oxidation peak to less positive values) which allows to improve the system performance. Although the performance reached with the nanocomposite-modified electrode is quite similar to the obtained with other systems where bioreceptors are used (ng mL^{-1} levels detected), the low specificity of this format of detection, limits its further application for the selective determination of AFs.

4.2. Enzymatic sensors

Enzymes are biological catalysts naturally produced by living organisms to accelerate biochemical reactions. This ability is exploited to detect AFs in two different formats: i) a catalytic pathway in which the AF is hydrolysed by an enzyme that produces an electroactive compound; and ii) a pathway based on the inhibition of the enzyme activity by the action of the AFs. As in the approaches discussed in previous section, nanomaterials are useful tools for electrode modification, with the aim of both improving the electronic transference and to create an environment favourable for the enzyme immobilization. However, the main limitation of these approaches is the lack of selectivity against other mycotoxins or molecules that can also inhibit the enzyme activity.

Actually, only one work is found in the last 5 years' bibliography, in which AFB_1 is detected thanks to its inhibitory effect on the activity of the enzyme acetylcholinesterase (AChE) using a titanium electrode, on which a nanocomposite made of TiO_2 nanotubes and gold nanoparticles is used for electrode modification and AChE deposition (AChE/AuNPs/TiO_2NTs) [110] (Fig. 5B). The photoelectrochemical response is due to the mercapto group present in the choline chloride generated in the decomposition of acetylcholine. These mercapto groups are easily oxidizable in the holes of TiO_2 nanotubes, thanks to the high surface area of the nanostructure. By increasing the concentration of AFB_1 the enzymatic reaction is inhibited and the photocurrent signal decreases, due to the decrease in the concentration of mercapto groups produced. With this design, a limit of detection at the ng mL^{-1} level is obtained, which does not improve the performance reached with the previously discussed approaches.

4.3. Molecularly imprinted polymer (MIP)-based sensors

A molecularly imprinted polymer (MIP) is a synthetic polymer with a specific recognition function for a specific analyte. MIPs are synthesized by the copolymerization of cross-linked and functional monomers in the presence of molecules (analyte) that act as template [111]. Once the synthesis reaction is complete, the templates are removed, leaving free gaps with multiple active sites corresponding to the spatial configuration of the model molecule in the polymer. In this situation, the polymer selectively identifies the template molecule and its analogue and can therefore be used as a recognition element in biosensors.

Conventional MIPs have important advantages, such as high specificity and sensitivity, ease of use, and low cost. Like aptamers, the fact that they are chemically synthesized (as alternative to receptors obtained by immunizing animals) provides this technology wide possibilities of modification to obtain the best possible benefits, low production costs and high reproducibility compared with antibodies. However, incomplete template removal and reduced use of active sites are undeniable limitations. For this reason, there is a growing interest in the development of improved MIPs.

An interesting recent approach is the proposed by Gu et al. [112], who synthesized a MIP in the presence of AuNPs for the detection of AFB_1. They used o-aminothiophenol (o-APT) as functional monomer, creating the film o-APT@AuNPs by electropolymerization on the electrode of a quartz crystal microbalance in the presence of the template, as shown in Fig. 5C. Another crucial issue is the development of a detection substrate under the MIP layer to further improve the detection ability. In this line, they used novel materials known as covalent organic frameworks (COFs), taking advantage of their predictable and well-ordered two-dimensional and three-dimensional porous structures. The introduction of the COFs-AuNPs substrate and the three-dimensional structure of the printed matrix provided the sensor with more recognition sites. Under optimal conditions, the proposed sensor showed a low detection limit (pg mL^{-1} levels) and acceptable recoveries in the analysis of real samples. A quite similar work in which organometallic structures are used is the recently reported by Jaffrezic-Renault's group [113], who developed for the first time a sensor with molecularly imprinted organometallic structures (MIP-MOFs) to determine AFB_1. To do this, first, an intermediate monolayer of p-aminothiophenol (PATP) is formed on a gold electrode by means of Au-S bonds. Subsequently, the organometallic structures containing the analyte are generated on the PATP-modified MIP by electropolymerization, making use of the previously synthesized PATP-functionalized gold nanoparticles (AuNP--PATP) and using AFB_1 as a template molecule. After removing the template molecule from the polymer matrix, a sensor was obtained with cavities in which AFB_1 will specifically bind through π-π interactions between aniline residues of the recognition holes and the analyte. This sensor results in a wide linear range and a low detection limit, in the fg mL^{-1} scale.

Finally, it is found in the recent bibliography the work reported by El Hassani et al. [114], who developed a MIP to determine AFB_2, based on two immobilization methodologies on a gold screen-printed electrode. One of the methodologies to modify the electrode was by electrodeposition of zinc oxide nanoparticles (ZnONPs) mixed with chitosan (Chi) and AFB_2, with a last extraction step of the AFB_2 template generating the analyte cavities. Alternatively, the second method consisted in the electrodeposition of the ZnONPs with Chi on the electrode with a subsequent stage of electropolymerization of a mixture of pyrrole (Py) and AFB_2 generating the MIP. Finally, the AFB_2 template was electrochemically removed, generating the active sites by over-oxidation of the PPy to introduce the oxygen-containing groups into the polymer structure. These two sensors were used to measure AFB_2 in milk samples at fg mL^{-1} levels.

It is also worthy to mention the combination of MIPs with photoelectrochemical detection [115], taking advantage of the characteristics of such detection mode, as detailed in previous sections.

As general remark, the sensitivity obtained the combination of MIPs and nanomaterial-based electrodes is the best among all the approaches summarized in this review, reaching limits of detection at the low fg mL^{-1} level (Table 3). However, important limitations make still limited the implementation of MIPs for routine AFs analysis. Such limitations are mainly related to the fact that that the interactions between the monomer and template molecule are weak. This means that a large amount of the functional monomer present in the polymer does not incorporate into the active binding site for the template molecule, which leads to a large number of low affinity and low selectivity binding sites. Important efforts for improving MIPs selectivity are expected for the upcoming years.

As in the case of the immunosensors, the strategies based on direct detection, enzymatic detection and MIP-based one have been only applied for the analysis of spiked samples, reaching good recovery rates.

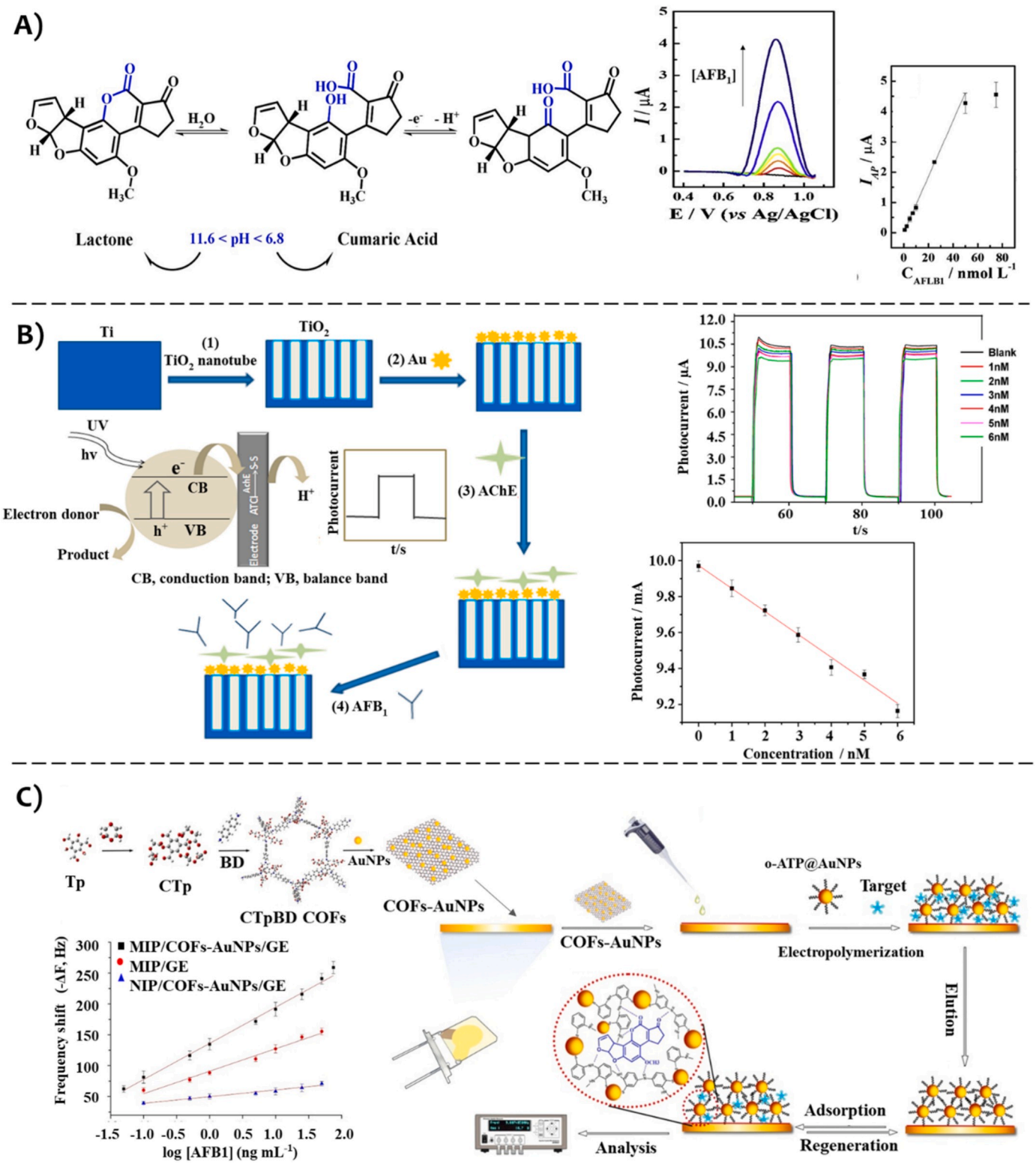


Fig. 5. Other electrochemical approaches based on nanomaterials for aflatoxins detection. A) Electrochemical oxidation of AFB_1 allowing its direct detection. LSV responses for different concentrations of AFB_1 and calibration plot. Adapted from Ref. [109] with permission. B) Enzyme inhibition-based biosensor for the detection of AFB_1. The decomposition of acetylcholine into choline and acetic acid generates mercapto groups that are photoelectrochemically detected. Calibration plots in buffer and in samples of sesame, walnut, pea and acetonitrile. Adapted from Ref. [110] with permission. C) Molecularly imprinted polymers (MIP)-based biosensor for the detection of AFB_1. Scheme of the COFs and COFs-AuNPs preparation, the QCM sensor and detection process. Adapted from Ref. [112] with permission.

Table 3
Other electrochemical approaches based on nanomaterials for aflatoxins detection reported in the period 2015–2021.

Sensor	Electrode	Technique*	Analyte	Lineal range	LOD	Spiked sample/ Recovery	Ref
Direct	AuNPs-GQDs/SPE	LSV	AFB_1	0.03–15.6 ng mL^{-1}	0.15 ng mL^{-1}	Malted barley/ 76–103%	[109]
Enzymatic	AChE/AuNPs/TiO_2NTs	PEC	AFB_1	0.3–1.8 ng mL^{-1}	0.1 ng mL^{-1}	-	[110]
Molecularly imprinted polymer (MIP)	o-ATP@AuNPs/AuNPs-COFs/ MIP	QCM	AFB_1	0.05–75 ng mL^{-1}	2.8 pg mL^{-1}	Peanut/87–94% Pistachio/95–102% Rice/87–96% Wheat/95–101%	[112]
	PATP-AuNPs/MOFs/MIP	LSV	AFB_1	1 fg mL^{-1} – 1 μg mL^{-1}	0.3 fg mL^{-1}	-	[113]
	ZnONPs/Chi/MIP	EIS	AFB_2	0.1–1000 fg mL^{-1}	0.2 fg mL^{-1}	Milk/Unspiked	[114]
	ZnONPs/Chi/PPy/MIP	DPV			0.6 fg mL^{-1}		
	CuO-g-C_3N_4/MIP	PEC	AFB_1	0.01 ng mL^{-1} - 1 μg mL^{-1}	6.8 pg mL−1	Maize/99–106%	[115]

*LSV: Linear Sweep Voltammetry; PEC: Photoelectrochemistry; QCM: Quartz Crystal Microbalance; EIS: Electrochemical Impedance Spectrometry; DPV: Differential Pulse Voltammetry.
AuNPs: gold nanoparticles; GQDs: graphene quantum dots; SPE: screen-printed electrode; AChE: acetylcholinesterase; TiO_2NTs: TiO_2 nanotubes; MIP: molecularly imprinted polymers; o-ATP@AuNPs: o-aminothiophenol functionalized AuNPs; COFs: covalent organic frameworks; PATP: p-aminothiophenol; MOFs: metal organic frameworks; ZnONPs: zinc oxide nanoparticles; Chi: chitosan; PPy: polypyrrole; CuO-g-C_3N_4: copper oxide-graphitic carbon nitride nanocomposites.

5. Conclusions and perspectives

The use of electrochemical biosensors based on nanomaterials for the detection of AFs, represents a vast emerging field of research. Metallic nanoparticles and carbon-based nanomaterials, are the main materials used for such purposes, whose role is both to increase the electronic transference and to improve the efficiency of the bioreceptor immobilization.

Antibodies and aptamers have been the main bioreceptors proposed for the specific recognition of AFs in spiked real samples, with good recovery rates. Molecularly-imprinted polymers (MIPs) are also emerging materials used for such purpose. In contrast, the low specificity of both the direct detection through their electroactive groups and the enzyme-based routes have much limited the development and implementation of such approaches.

The high specificity of antibodies together with the well-known procedures for their conjugation and immobilization on nanomaterial-modified electrodes, have make the immunosensing routes as the most widely used so far. However, the relative high cost and low availability of monoclonal antibodies still remains an important limitation for the immunosensors implementation for routine AFs analysis.

Regarding the use of aptamers, in spite of their advantages over antibodies, in terms of cost, stability and reproducibility, their lower specificity and lower availability are still key limitations for their implementation.

In the case of the MIPs, affinity and selectivity improvements are still required for considering them as a reliable alternative to antibodies and aptamers.

Among the different assay formats, label-free approaches, using both antibody and aptamer receptors are predominant, benefitting from the simplicity speed and low cost of analysis. However, and despite the efforts in the integration of nanomaterials for improving the sensitivity of these approaches, the limits of detection reached are typically at levels of ng mL^{-1}, which in some cases is not enough for fulfilling the legislation requirements. This is of special relevance when extraction methodologies involving sample dilution are needed. Another important limitation is related to the unspecific signals due to electrode fouling, what limits its implementation for the analysis of samples with complex matrixes.

The selectivity and sensitivity/detection limit required, and the availability of specific bioreceptors are the main parameters that should define the detection strategy more suitable for each particular application.

Regarding the applicability for real sample analysis, most of the approaches found in the bibliography so far have been applied for the analysis of spiked samples (i.e. corn, maize, rice, wheat, milk, olive oil, etc.) with good recovery rates. The need of extraction methodologies still represents a limitation for unspiked sample analysis. Efforts in the integration of AFs extraction/preconcentration methodologies (i.e. by using immunoaffinity columns) are expected for the upcoming years.

As far as we know, none of the reported electrochemical biosensors for AFs detection are neither commercially available nor reached the laboratory for routine use yet. In this line, some important issues should be solved for the implementation of such sensing systems for routine analysis, as alternative/complementary to centralized laboratory-based methods (HPLC-MS/MS; GC-MS/MS). Long-term stability of the biosensors is a crucial issue that is not addressed in most of the reviewed works. Moreover, as previously mentioned, AFs extraction from food samples still requires quite complex methodologies involving the use of solvents that in some cases are not compatible with both the bioassay and the electrochemical detection. In addition, multi-detection abilities should be strongly required for real applications in AFs screening, as shown in recent reports [116,117]. Important efforts related to these issues should be the next at the current state of the before crossing the so-called “valley of death”, from basic research to the real implementation for routine use at the laboratory.

Overall, the higher potentiality of electrochemical biosensors for AFs is in the “in field” analysis, in our opinion. We envisage the implementation of such devices for a decentralized screening, which would minimize the number of samples sent to head laboratories for being analysed with the above mentioned well-stablished methodologies. The combination of miniaturized electrochemical transducers such as screen-printed electrodes (SPEs) with cheap and portable electrochemical instruments make altogether ideal for such applications. The most challenging issue here is probably related to the sampling, incubation and washing steps required in most of the bioassays, which limits their use by non-skilled people and consequently their commercial implantation. The combination with microfluidics seems to be of key relevance for solving this issue, so high efforts in this sense are also previewed for the upcoming years.

CRediT authorship contribution statement

Beatriz Pérez-Fernández: Bibliographic search, Writing – original draft. **Alfredo de la Escosura-Muñiz:** Writing – review & editing, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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