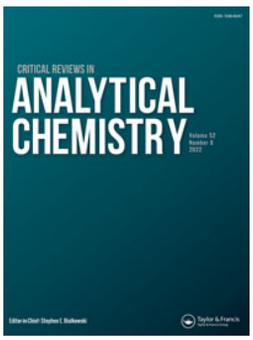

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Novel Sensor Approaches of Aflatoxins Determination in Food and Beverage Samples

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ABSTRACT

The rapid quantification of toxins in food and beverage products has become a significant issue in overcoming and preventing many life-threatening diseases. Aflatoxin-contaminated food is one of the reasons for primary liver cancer and induces some tumors and cancer types. Advancements in biosensors technology have brought out different analysis methods. Therefore, the sensing performance has been improved for agricultural and beverage industries or food control processes. Nanomaterials are widely used for the enhancement of sensing performance. The enzymes, molecularly imprinted polymers (MIP), antibodies, and aptamers can be used as biorecognition elements. The transducer part of the biosensor can be selected, such as optical, electrochemical, and mass-based. This review explains the classification of major types of aflatoxins, the importance of nanomaterials, electrochemical, optical biosensors, and QCM and their applications for the determination of aflatoxins.

KEYWORDS

Aflatoxins; sensor; food samples; analysis; determination

Introduction

Food safety, clean, and contamination-free food content is one of the most important issues in today's developing and growing world. Contamination of agricultural products by various toxic factors affects humans and all the living organisms in the ecosystem. The decrease in safe food and water resources and the fact that many underdeveloped countries struggle to access these resources bring socioeconomic problems globally.[1,2] One of the most significant contaminants that threaten the health of living things are mycotoxins which are secondary metabolites produced by fungi.[2] Currently, more than 400 mycotoxins have been identified, and they are responsible for the contamination of agricultural crops worldwide, as stated by The Food and Agriculture Organization (FAO).[3] Mycotoxins cover a wide group of compounds (aflatoxins, ergot alkaloids, ochratoxins, etc.), and they have serious toxicity, including carcinogenicity, immunosuppression, teratogenicity, and mutagenicity.[4]

Among all mycotoxins, aflatoxins which are produced by the Aspergillus genus of fungi, stand out with their high toxicity and carcinogenicity.[5] Aflatoxins were firstly recognized in the 1960s, and since then, 18 different types of aflatoxins have been discovered (AFB1, G1, M1, B2, etc.). The double furan ring and coumarin structure, which are common in the chemical structure of aflatoxins, are the structures responsible for the toxic effect.[6] Due to the prevalence and highly toxic effects of aflatoxins, international authorities took significant steps, and they were classified as Group 1 carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO).[7] Additionally, there are Maximum Residue Levels (MRL) set by the European Commission for different types of aflatoxins such as AFM1 and AFB1.[4]

The occurrence of aflatoxins can be seen in a wide variety of food products: cereals, nuts and seeds products, milk and dairy products, etc. By this means, humans are exposed to aflatoxins, even in small amounts, cumulatively.[3,6] Considering common occurrence and related health risks of aflatoxins, the development of accurate, precise, highly sensitive, and reliable sensors for the determination of aflatoxins in both food products and biological fluids has great importance. Therefore, numerous studies are available in the literature utilizing different methods and techniques. Well-established methods for aflatoxins are mostly chromatography-based combined techniques and enzyme-linked immunoassays. Despite the reliability advantage, these methods lack affordability and easy application.[3]

Nowadays, researchers focus on novel sensor approaches that can offer user-friendly, rapid, effective, selective, and sensitive analysis. In this context, biosensors provide a superior approach in many aspects for the analysis of various analytes.[3,4] Differentiation of the biorecognition element part of the biosensors with components such as an

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enzyme, molecularly imprinted polymer (MIP), and aptamer provides high specificity toward the target analyte. In addition, according to the desired analysis and analyte, the transducer part of the biosensor can be selected, such as optical, electrochemical, and mass-based.[1,8,9]

For the determination of aflatoxins, biosensor applications have been a popular topic recently. When the literature is examined, it can be seen that there are several review studies focusing on the determination of aflatoxins. Liu et al.[3] discussed the electrochemical biosensors for aflatoxin detection.[10] They focused on the ratiometric, electrochemical, electrochemiluminescence, and photoelectrochemical methods and all types of aflatoxins in their work. On the other hand, some studies focused on the detection of a specific type of aflatoxin. Vaz et al.[11] summarized the sample preparation and determination methods for aflatoxin M1, while Xue et al.[5] overviewed the recent works for aflatoxin B1 detection using nanotechnology and nanomaterials. In another review study by Gurban et al.[12] electrochemical biosensor platforms for the detection of aflatoxin M1 in milk and dairy products were evaluated. Another method-based review example is the study by Wu et al.,[13] which provides an overview of the determination of aflatoxins from nut and dried fruit samples by spectroscopic and imaging techniques.

This review explains the novel and the most recent sensor approaches and applications for the determination of aflatoxins in food samples.

Major types of aflatoxins

Aflatoxins, low molecular weight, highly toxic mycotoxins are produced by the Aspergillus genus as a seconder metabolite, especially by Aspergillus flavus and Aspergillus parasiticus and they are widely found in fungus contaminated crops and foods[14–16] Soil-originated Aspergillus contamination in crops can become a significant risk due to wrong postharvest application which causes an increase in microbial growth, especially in figs, peanuts, cereals, and corn.[16] Moreover, when cows are fed with such food, toxins pass into the milk and cause secondary contamination in milk and dairy products.[17] Both humans and animals are directly exposed to these toxins by the consumption of contaminated foods.[14,16] It was reported that consuming aflatoxin-contaminated food is one of the reasons for primary liver cancer and induces some tumors and cancer types such as renal cancer or rectal cancer.[18]

More than 20 types of aflatoxins (Tables 1 and 2) are known, which all have difuran rings attached to the coumarin (Figure 1).[14,16] Around the coumarin nucleus, besides

Table 1. Classification of aflatoxins in terms of sources and contaminated products[15].

Aflatoxin	Source	Frequently Contaminated Products
Difurocoumarocyclopentenone		
Aflatoxin B1	*Aspergillus*; Section *Flavi* Section *Ochraceorosei* Section *Nidulantes*	Cereals, oilseeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products
Aflatoxin B2	*Aspergillus*; Section *Flavi* Section *Ochraceorosei*	Cereals, oilseeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products
Aflatoxin $B2_a$	Hydroxylated metabolite of B1	NA
Aflatoxin M1	Hydroxylated metabolite of B1	Milk and dairy products, Meat products
Aflatoxin M2	Hydroxylated metabolite of B2	Milk and dairy products, Meat products
Aflatoxin $M2_a$	Hydration of the terminal furan ring of aflatoxin M1	Milk and dairy products,
Aflatoxin P1	Demethylated metabolite of aflatoxin B1	Excreted in the humans and animals' urine
Aflatoxin Q1	Hydroxylated metabolite of aflatoxin B1	Assumed to be inedible parts of bovine fed on aflatoxin B1-contaminated feed
Aflatoxin $Q2_a$	Acid hydration of aflatoxin Q1	NA
Aflatoxicol (R_0)	Metabolite of aflatoxin B1 formed by a reversible reduction of the pentanone group.	Mainly avian products. Dairy products.
Aflatoxin M1	Reduced metabolite of aflatoxin B1, aflatoxin R0, or aflatoxin M1	Milk and dairy products
Aflatoxicol H1	Reduced metabolite of aflatoxin B1 and aflatoxin Q1	Milk and dairy products
Difurocoumarolactone		
Aflatoxin G1	*Aspergillus* Section *Flavi*	Cereals, oilseeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products
Aflatoxin G2	*Aspergillus* Section *Flavi*	Cereals, oilseeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products
Aflatoxin $G2_a$	Hydroxylated metabolite of aflatoxin G1	NA
Aflatoxin GM1	Hydroxylated metabolite of aflatoxin G1	Milk and dairy products
Aflatoxin GM2	Hydroxylated derivative of aflatoxin G2	Milk and dairy products
Aflatoxin $GM2_a$	Metabolite of aflatoxin GM1in the liver of mammals	Milk and dairy products
Parasiticol (aflatoxin B3)	A metabolite of aflatoxin G1 from the biodegradation	Cereals, oilseeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products
Others		
Aspertoxin	*A. flavus and A. parasiticus*	Contaminated vegetable products

Table 2. Physicochemical and toxicological properties of the major aflatoxins[15].

Aflatoxin	Mw (g/mo)	Formula	Health Effects
Aflatoxin B1	312.063	$C_{17}H_{12}O_6$	Hepatotoxicity, genotoxicity, carcinogenicity, immuno-toxicity, teratogenicity
Aflatoxin B2	314.079	$C_{17}H_{14}O_6$	Week mutagenicity, hepatotoxicity, and carcinogenicity
Aflatoxin B2a	333.074	$C_{17}H_{14}O_7$	Low toxicity (200-fold less than B1)
Aflatoxin M1	328.058	$C_{17}H_{12}O_7$	Hepatotoxicity, nephrotoxicity, carcinogenicity
Aflatoxin G2	330.074	$C_{17}H_{14}O_7$	Low toxicity, no evidence for carcinogenicity in animals
Aflatoxin G2a	346.069	$C_{17}H_{14}O_8$	Low toxicity to inactive (a detoxified form of G1)

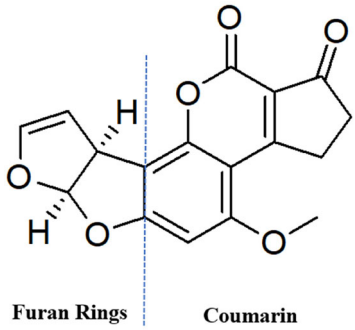

Figure 1. Chemical structure of Aflatoxins.[14]

difuran rings, either a pentene ring or a six-membered lactone ring is also attached in their chemical structure (Figure 2).[15] According to this side group, aflatoxins are divided into two main groups; (Figure 2A) aflatoxins that have pentene ring, difurocoumarocyclopentenones, which are B type aflatoxins and derivatives, (Figure 2B) aflatoxins that have lactone, difurocoumarolactones which are G type aflatoxins like G1, G2, M1 and M2.[15] As it is represented in Figure 2, the Coumarin core in the (green) center and the bifuran ring (Blue) in the left side is common in both aflatoxin types. However, the moiety in the right site represented in red in Figure 2 can vary and can be either cyclopentene or a six-membered lactone ring.[15] It is important to underline that the C8 = C9 double bond in the bifuran rings plays a major role in the toxicity of aflatoxins.[15]

Although there are different methods for aflatoxin detection, using absorption and emission spectra at 360 nm is the most common technique among them. Besides the differences in their chemical structures, another important difference between aflatoxin is the fluorescence that they exhibit under UV irradiation since they are highly fluorescent.[19] While B-type toxins radiate blue fluorescence at 425 nm, G toxins radiate green fluorescence at 540 nm under UV irradiation[20] (Figure 3).

Aflatoxins' molecular weights range from 312 to 346 g/mol and they are soluble in organic solvents.[14,16,21] The most important known aflatoxins are B1, B2, G1, and G2 due to their high toxicities and high incidence.[16] Including the B and G family, 13 types of aflatoxins are produced by the fungus. However, some types of aflatoxins are not directly produced but they are derived from other aflatoxins.[15] Aflatoxin M1, which originates from enzymatic hydroxylation of B1 in the cows' digestion system, is another common, important aflatoxin type that also has a hepatic and carcinogenic effect.[17,22] Thus especially aflatoxin B1, B2, G1, G2, and M1 are of most concern to the economy and public health.[15] Physicochemical and toxicological properties of the 6 major aflatoxins can be found in Table 2. According to International Agency for Research on Cancer (IARC),[23] due to their carcinogenic and toxic effects, aflatoxin B1 is classified as Group 1 carcinogenic, and the rests are Group 2B[14,23] (Figure 4).

After consumption of aflatoxin-contaminated forage by cows, Aflatoxin B1 is converted to Aflatoxin M1 by enzymatic hydroxylation at the 9a position. Thus the only structural difference between B1 and M1 is the presence of the hydroxyl group at the 9a position, which causes a difference in toxicity level.[17]

The importance of nanomaterials on biosensors for detection of aflatoxins

Concerns about food safety, quality, and control increased the need for new technological approaches based on biosensors for contamination monitoring across the food chain. Biosensors have proven their capacity to deliver on-site testing results with short analysis time, sensitivity, selectivity, robustness, user friendly, and labor-saving. In recent years, the evolution of biosensor devices for the determination of aflatoxins has piqued scientists' interest, with a variety of devices being developed and described in the research studies. The development of nanotechnology and its effect on the growth of ultrasensitive aflatoxins analysis is benefiting from breakthroughs in the production of nanomaterials for sensors.

To improve sensitivity and ease of detection, nanomaterials such as different shapes and structures of gold nanoparticles, magnetic particles, quantum dots, and carbon materials especially carbon nanotubes (CNTs), graphene, and its derivatives have been widely used for aflatoxin analysis.[6] These nanomaterials are used as biomolecule immobilization supporters and electroactive or optical signal labels to attain amplification.[6] Many nanoparticle-based analytical detection methods have been created using nanoparticles for the detection of aflatoxins. Generally, these methods are based on electrochemical, immunosensors, and optical detection.

Varlamova et al.[24] proposed amperometric biosensors with electrode modification based on graphene oxide (GO) and CNTs and gold nanoparticles (Au-NPs) in chitosan. These modifications by nanoparticles maintain high electroactivity and lead to obtaining good analytical results in a concentration range of 1×10^{-11}–1×10^{-6} M with a detection limit of 5×10^{-12} M for determining aflatoxin M1.

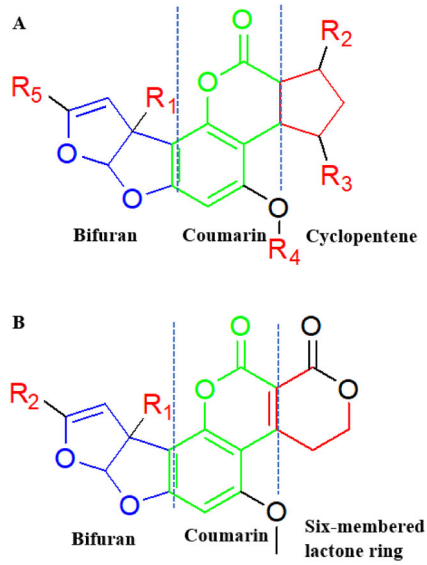

Figure 2. Difurocoumarocyclopentenone type Aflatoxin **(A)**, Difurocoumarolactone type Aflatoxin **(B)**.[15]

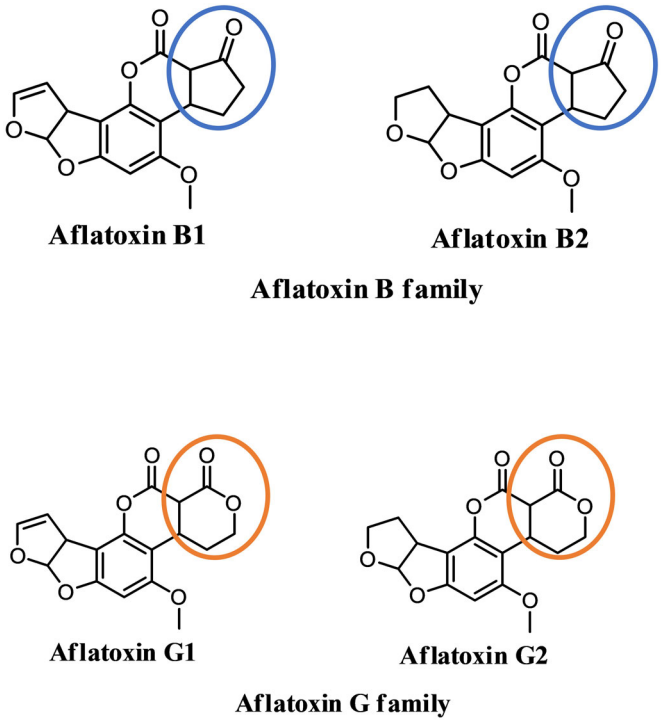

Figure 3. Chemical structure of Aflatoxins of B family (B1 and B2) and G family (G1 and G2). The moiety in the blue or red Circle shows the difference between the two groups.[14]

Also, this developed sensor can be used to determine aflatoxin M1 in milk, kefir, and cottage cheese.

Another study by Kordasht et al.[25] developed an aptasensor with nanosilica and gold nanoparticles for the detection of aflatoxin M1 shown in Figure 5. These nanomaterials are electrodeposited on the surface of a glassy carbon electrode, and quantification of aflatoxin M1 is completed by cyclic voltammetry and differential pulse voltammetry. The fabricated aptasensor shows a low limit of detection (10 fM), the calibration curve is linear between

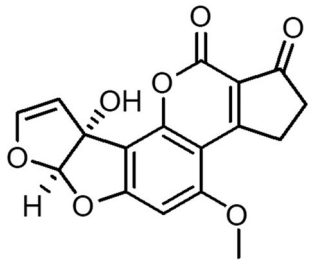

Figure 4. Aflatoxin M1 chemical structure.[17]

0.1 µM to 10 fM, and can be used in the analysis of milk samples.

CNTs are commonly used to develop electrochemical biosensors to enhance analytical responses due to their large surface area, mechanical strength, good flexibility, and chemical stability; this may improve the electrochemical analysis of different biomolecules that are widely used in immunosensors.[6] Singh et al.[26] developed an electrochemical immunosensor based on carboxylated CNTs for the detection of aflatoxin B_1 what was electrochemically deposited onto indium tin oxide (ITO) glass electrode. Functionalization of MWCNTs with carboxyl group covalently bonded to aminated antibodies strongly results in the sensitive detection of aflatoxin B1, and a stable biosensor can be obtained, as shown in Figure 6. The developed immunosensor has a low detection limit (0.08 ng mL^{-1}) in the linear detection range of 0.25–1.375 ng mL^{-1}.

Besides these nanomaterials, more than one nanomaterial can be used together to enhance the electrochemical performance of biosensors. For instance, Linting et al.[27] described an immunosensor that depends on graphene/conducting polymer/Au NPs/ionic liquid composite film for aflatoxin B1 determination. The usage of graphene and Au NPs provides fast electron transfer. The conducting polymer is covalently attached to the antibody. The mixture of ionic liquid with chitosan also creates a safe environment for antibody binding. This sensor has a very low detection limit (1 fM) and is in the linear range from 3.2 fM to 0.32 pM, also, this method can be applied for analysis of real spiked food samples.

Because of their distinctive optical and electrical properties, nanomaterials-based colorimetric immunoassays have drawn more attention for detecting various biomarkers. Althagafi et al.[28] developed selective optical sensor based by using silica nanoparticles decorated with gold nanoparticles for detection of different aflatoxins (Afs). Firstly, they prepare mesoporous silica particles (m-SiNPs) with sol-gel method then decorated with gold nanoparticles (Au NPs), then modified with Afs antibodies (AFs-Ab) to create AFs-Ab/Au NPs@m-SiNPs. When aflatoxins and AFs-Ab/Au NPs@m-SiNPs get interaction, this results in color change from pink to violet. This developed immunoassay has wide concentration range from 1 ng·mL^{-1} to 75 ng·mL^{-1} and low LOD value (0.16 ng·mL^{-1}). This designed immunoassay was applied to raw foodstuff samples.

Nanomaterials are used to fabricate electrochemical biosensors to increase analytical responses because of their unique benefits like wide surface area, simple synthesis, and

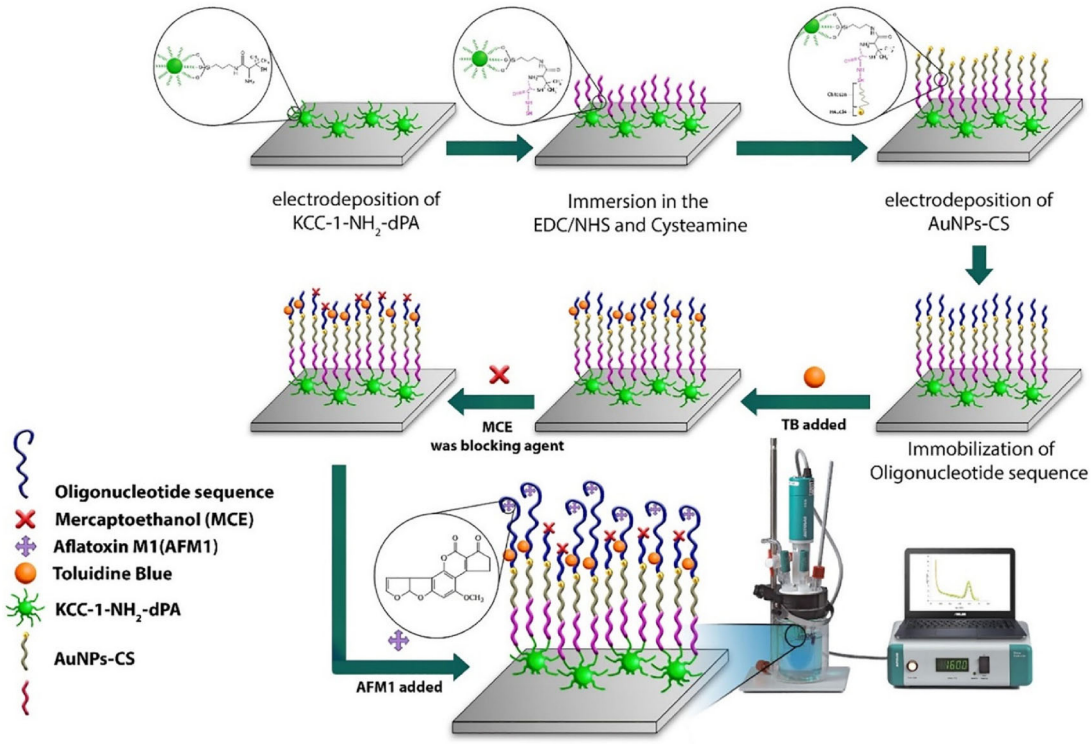

Figure 5. Schematic representation of developed aptasensor for detection of aflatoxin M1. Reproduced with permission from Elsevier.[25]

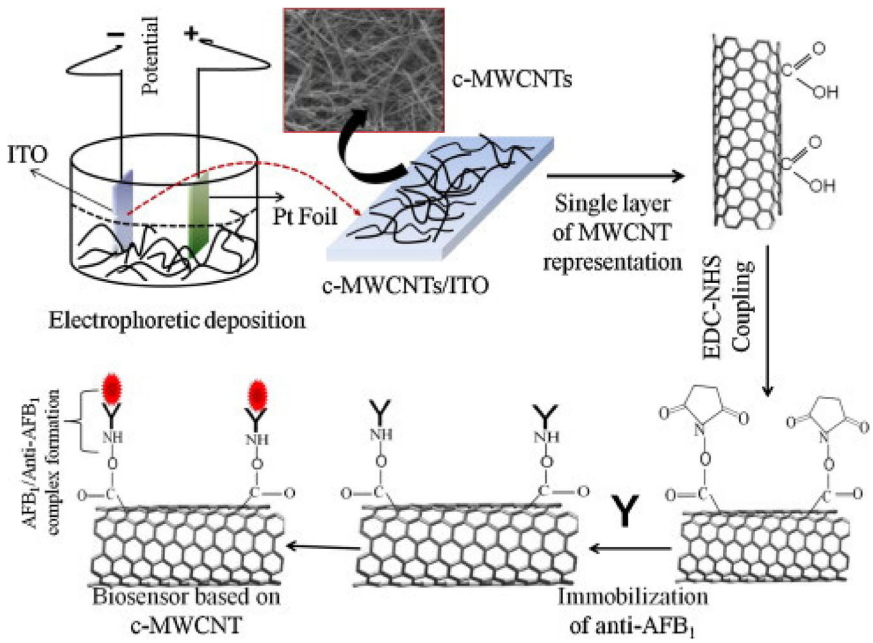

Figure 6. Schematic representation of fabricated carboxylated carbon nanotubes based electrochemical immunosensor for detection of aflatoxin B1. Reproduced with permission from Elsevier.[26]

great electrochemical performance. They are mostly used as electrode modification agents.

Application of biosensors for determination of aflatoxins

An extensive literature review was conducted to investigate and monitor AFB1-contaminated food. Several methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectroscopy, enzyme-linked immune-sorbent assay (ELISA), and electrochemical and optic sensor have been used for detecting and quantifying aflatoxins in foods and beverages.[29] The most commonly used chromatography techniques for analysis of aflatoxins are TLC, HPLC, and gas chromatography (GC). Although many of the chromatographic techniques are very sensitive, they require trained skilled technician,

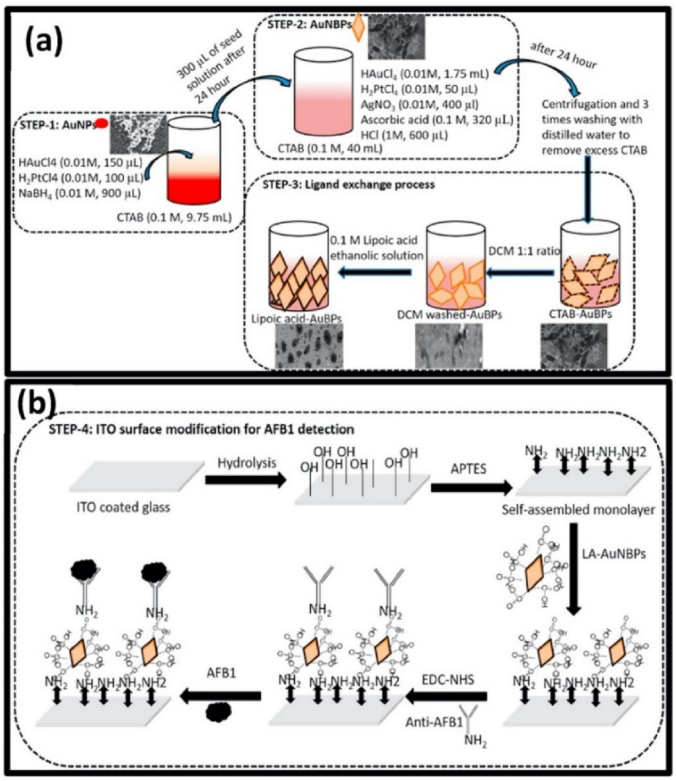

Figure 7. Schematic depiction of a) synthesis process of AuNBPs and lipoic acid-AuNBPs and b) ITO surface modification. Reproduced with permission from Elsevier.[9]

cumbersome pretreatment of sample, and expensive apparatus/equipment. On the other hand, it has been observed that nanomaterial-based biosensors are frequently used. For the detection of AFB1, transducers giving different analytical signals such as current, potential, conductivity, and impedance were used.[30,31] Also, biosensors used for AFB1 detection were classified as electrochemical and potentiometric biosensors depending on the type of transducer element used. In addition, although biosensors were effective in the detection of AFB1, nanomaterial-based biosensors showed better analytical performance. The advantages and disadvantages of different biosensors used for AFB1 detection in food samples will be discussed in this section. The optical and electrochemical sensor systems have been demonstrated as excellent alternatives in replacing the traditional sensing and analytical systems. These optical and electrochemical sensors have advantages like portability, shortened analysis time, ease of operation, novice-friendly and direct analysis with no sample preparation procedures. Thus, the nanomaterial-based optical and electrochemical sensing platforms have been acknowledged as reliable tools for automated on-site analysis of aflatoxins in food processing and manufacturing industries.

Electrochemical biosensing devices are frequently used in the evaluation of food samples due to their low cost, short analysis time, and sensitive and portable nature.[32] AFB1 monitoring in food samples is performed using different electrochemical techniques such as amperometry, potentiometry, and impedance spectroscopy according to the type of analytical signal.[33] Depending on the amount of AFB1 in food samples, interactions between the biorecognition element cause changes in signal types in the specified techniques, and analyzes are performed by evaluating the data obtained.[34]

Electrochemical devices that can sensitively and selectively measure current differences at constant potential are called amperometric biosensors. This current value changes depending on the AFB1 concentration in the food samples. Although more than one electrode system is used, the best analytical performance has been obtained in three-electrode systems.[32] Especially, the auxiliary electrode enhances the surface area by making a positive contribution to the electron kinetics during the detection of AFB1 in food samples.[32] Another commonly used electrode system is the two-electrode system. Li et al.,[35] developed amperometric biosensors for the immobilization of aflatoxin oxidase using multi-walled carbon nanotubes (MWCNTs) for AFB1 detection in foods. For this, cyclic voltammetry, was used, and a platinum electrode was preferred as the working electrode. The linearity range of the developed sensor was 0.0032–0.720 µM, and the LOD was 1.6 nM. In food samples, the AFB1 very short analysis time of 44 seconds and high sensitivity were the most important advantages of the sensor.

Impedimetric biosensors are based on electrical conductivity and capacitance, and electrochemical response occurs after the interaction of AFB1 in food samples with biorecognition elements.[36] These sensors monitor changes in impedance by immobilizing AFB1 on the working electrode by the biorecognition element.[37,38] Bhardwaj et al.,[9] developed an electrochemical impedimetric biosensor for AFB1 detection in maize samples using gold nano-bipyramids (AuNBPs). A two-step seed-mediated growth process was used to synthesize these AuNBPs (Figure 7). The surface of the coater was then modified using lipoic acid (Figure 7a). Self-assembled 3-aminopropyl triethoxysilane (APTES) film was used to confine these AuNBPs on indium in oxide (ITO) glass (Figure 7b). Impedimetric measurement of AFB1 was performed using the obtained ITO electrode and anti-AFB1. The LOD value and linear dynamic range (LDR) of this biosensor were found to be 100 µM and 100 µM–25 nM, respectively. Furthermore, in AFB1-contaminated maize samples, this biosensor was exhibited superior recoveries ranging from 95 to 100%.

The potentiometric biosensor, when the current is kept constant, identifies the analyte with electrical potential measurement.[8,39,40] Li et al.[40] developed a potentiometric immunosensor for detecting AFB1 in peanut samples. This biosensor was constructed by coupling a combination of anti-AFB1 and BSA to the surface of functionalized polyclonal anti-AFB1 with AuNPs and a glassy carbon electrode. Competitive immunoreactions occurring at the working electrode were used to detect AFB1 utilizing the developed biosensor. Relying on the competitive binding of the analyte (AFB1) and the anti-AFB1-BSA complex with polyclonal anti-AFB1, the introduction of AFB1 results in competitive antigen-antibody immunological reactions. As a result of these immunoreactions, the surface potential and charge of

the working electrode altered. This change in working potential was related to the AFB1 concentration of the working electrode, which reduced the binding capacity of polyclonal anti-AFB1-AuNPs. The LDR and LOD of the developed potentiometric immunosensor were found 0.1–5.0 g/kg and 87 ng/kg, respectively.

Electrochemical biosensors for determination of aflatoxins

Electrochemical methods have important features such as portable equipment, low technical requirements, eco-friendliness, it is affordable, and has a quick response.[34] The combination of biosensors and point-of-care (POCs) diagnostic devices would provide a promising platform for the accurate monitoring of aflatoxins in clinical and non-clinical fields, such as agricultural, food, and beverage industries or food control processes.[41] Thus, many researchers focused on fabricating electrochemical biosensors. In electrochemical transducer systems, an electrical signal in terms of potential, current, or impedance is generated due to the interaction of analytes and biorecognition elements at the electrode surface.[34,42] Therefore, the electrochemical sensor can be sensitive enough to measure the trace level analytes in wide LDR. It is highly selective for the target analyte of interest and is stable over a long time. According to the biorecognition element, the electrochemical biosensors are divided into aptamers-based biosensors, antibodies-based biosensors, and MIP-based biosensors in this review. The specifications of the analyte, sensor, LDR, LOD, recovery, and sample application of biorecognition elements are listed in Tables 3–5, respectively.

Aptamers-based biosensors

The aptamer-based electrochemical biosensor systems have drawn considerable interest in the analysis of AFB1.[43] Aptamers are used in biosensor applications. Aptamers are identified as single-stranded nucleic acid (index-enriched ligand) or peptides that can specifically bind small molecules with high affinity and specificity, similar to antigen-antibody interactions. The systematic evolution of ligands by exponential enrichment (SELEX) is used for the selection process of oligonucleotide probes.[44] The SELEX process is a very important tool for obtaining the best biorecognition probe. Aptamers-based biosensors have many advantages (in contrast to traditional electrochemical biosensors), such as easy preparation, high specificity (strong affinity), stability, and determination of the wide range of target molecules.[44] Aptamers-based biosensors for AFB1 comprise various formats by using different aptamers such as label-free aptasensor, electrochemiluminescence aptasensor, photoelectrochemical aptasensor, ratiometric electrochemical aptasensor. Table 3 summarizes the analysis of aflatoxins in electrochemical sensor applications involving aptamers. In this section, we have reviewed several promising aptasensor systems.

Zhong et al. constructed a label-free electrochemical aptasensor capable of sensitively detecting aflatoxin B1 (Figure 8). The electrochemical aptasensor was fabricated by electrodepositing gold nanoparticles on the glassy carbon electrode modified with Zeolitic imidazolate frameworks (ZIF-8). Based on this methodology, an electrochemical aptasensor has been constructed with the LDR of 10.0–1.0×10^5 pg/mL of AFB1 and LOD of 1.82 pg/mL. This aptasensor has been successfully applied for the analysis of AFB1 in corn oil and peanut oil samples.[45]

Mousavi Nodoushan et al. offered a high specificity, fast and accurate sensing method through the electrochemical aptasensor of AFB1 based on graphene oxide and gold nanowires.[108] Graphene oxide and gold nanowires were used to create a synergistic effect. The LDR was 5.0–750.0 pM, and LOD 1.4 pM. The developed aptasensor was successfully applied in pistachio samples with a high recovery percentage and low RSD% value.[72]

Antibodies-based biosensors

Antibodies-based biosensor is also known as immunosensor. The interaction between analytes and biorecognition elements (antibodies) takes place on the transducer (electrode). Electrochemical immunosensors have achieved significant progress in various applications due to the good interaction between specific antigens and antibodies as well as the high sensitivity of electrochemical methods. The various applications are summarized in Table 4.

Shi et al. developed an electrochemical immunosensor for the AFB1 detection using reduced graphene oxid (Figure 9). In this assay, 4-aminobenzoic acid was used to reduce graphene oxide by means of an epoxy ring-opening reaction. Au-poly PABA (PPABA)-r-GO nanohybrids were formed with Au NPs. These nanohybrids were dropped on the GCE surface. Therefore, AFB1 antibodies were covalently linked with carboxyl groups of nanohybrids. Au NPs simultaneously bond AFB1 antibodies in a synergistic reaction. Bovine serum albumin was used for blocking nonspecific interaction. The electrochemical impedance spectroscopy was performed using $Fe(CN)_6^{3-/4-}$as redox marker. Impedimetric response was obtained in the LDR of 0.01–1 ng/mL and 1–25 ng/mL with LOD of 0.001 ng/mL. The applicability was evaluated in vegetable oil samples.[103]

Pei et al. developed a photoelectrochemical (PEC) immunosensor for AFB1 detection using gold nanoparticles (Au NPs) as a highly sensitive platform. The sensitive platform can immobilize antibodies via Au NPs, and enhance the separation of electron-hole pairs due to its good energy band matching efficiency. The obtained PEC immunosensor exhibits a good LDR from 1.0 pg/mL to 100 ng/mL, and LOD 0.33 pg/mL. This immunosensor also exhibits satisfactory reproducibility, stability, and specificity. Moreover, the immunosensor successfully detects AFB1 in peanut samples.

MIP-based biosensors

Molecularly imprinted polymer (MIP) designs excellent molecular recognition sites that mimic several biological

Table 3. The summary methods for monitoring of aflatoxins using aptamers based biosensors.

Analyte	Sensor	Linear Range	LOD	Recovery	Sample Application	Ref.
AFB1	Electrochemical Aptasensors	10.0–1.0 × 10^5 pg/mL	1.82 pg/mL	93.49–106.9%	corn oil and peanut oil	[45]
AFB1	Electrochemical Aptasensors	0.1–1000 pg/ mL	0.032 pg/mL	96.3–102.1%	peanut	[46]
AFM1	Electrochemical Aptasensors	0.5–800 ng/L	0.3 ng/L	90–108%	milk	[47]
AFB1	Electrochemical Aptasensors	100 pg/mL–100 ngmL	38.8 pg/mL	93–106.5%	corn, wheat, peanut, and rice	[48]
AFB1	Electrochemical Aptasensors	5.0 × 10^{-3}–150.0 ng/mL	1.0 × 10^{-3} ng/mL	98.4–101.3%	rice flour	[49]
AFB1	Electrochemical Aptasensors	0.01 μg/L–20 μg/L	9.4 × 10^{-4} μg/L	88.4–102.0%	cereals	[50]
mycotoxin, ochratoxin A (OTA).	Electrochemical Aptasensors	1.56–400 ng/mL	0.6 ng/mL	96.25–97%	rice floor	[51]
AFB1	Electrochemical Aptasensors	100 aM–100 pM	6.75 aM	91.0–99.7 %	beer samples and the food	[52]
AFB1	Electrochemical Aptasensors	0.1–75 μg/L	31 ng/L	96.05–104.45 %	rice and corn flour	[53]
AFM1 and AFB1	Electrochemical Aptasensors	30–900 pg/mL	0.9 nM	95.4–108.1%	milk and serum	[54]
AFB1	Electrochemical Aptasensors	1.0 × 10^{-3} −200.0 ng/mL	8.3 × 10^{-4} ng/mL	97.8–105.5%	wheat flour	[55]
AFB1	Electrochemical Aptasensors	0.01–50 ng/ mL	3 pg/mL	98.5–110%	grape juice milk soy milk	[56]
ZEN	Electrochemical Aptasensor	5.0 × 10^5 ng/mL–50 ng/mL	0.013 pg/mL.	89–102%	corn and beer.	[57]
AFB1	Ratiometric Electrochemical Aptasensor	0.003–3 pg/ mL	0.00088 pg/mL	105–114%	peanut	[58]
AFB1	Reusable Electrochemical Aptasensor	0.01 − 100 ng/ mL	0.01 ng/mL	85–106 %	peanut	[59]
AFB1	Label-Free Electrochemical Aptasensor	3 ag/mL − 1.9 μg/mL	0.9 ag/mL	97.2–104.4%	wheat flour	[60]
AFB1	Electrochemical Aptasensor	1–100 pg/mL	0.6 pg/mL	105–109%	milk	[61]
AFB1	Hairpin DNA-Based Dual-	10–3000 pg/mL	4.3 pg/mL	94.0–103.5%	corn and wheat	[62]
ochratoxin A (OTA)	Ratiometric Electrochemical Aptasensor	30–10000 pg/mL	13.3 pg/mL	93.5–105.0%		
AFB1	Aptamer Electrochemical Sensor	8 pM–25 nM 25 nM–3 μM	6 pM	–	white wine and milk	[63]
AFB1	Colorimetric/ Electrochemical Dual-Channel Aptasensor	0.05–100 ng/ mL	0.43 pg/mL	92.6–97.5%	corn	[64]
AFB1	Competitive Impedimetric Aptasensors	0.05–3 pg/mL	–	92.5-–108%	beer and serum	[65]
AFB1	Electrochemical Aptasensors	1.0 × 10^{-13}–1.0 × 10^{-8} M	5.5 × 10^{-14} M	96–109%	peanut	[2]
AFB1	Electrochemical Aptasensors	0.001–0.1 ng/ mL	0.002 ng/mL	94.5–103.3%	Oil, soy sauce	[66]
AFB1	Ratiometric Electrochemical Aptasensor	0.05–20 ng/mL	0.016 ng/mL	86.5–112%	peanut	[67]
AFM1	Label–Free Electrochemical Aptasensor	1.0 × 10^{-2} −80.0 ng/mL	2.0 × 10^{-3} ng/mL	93–108%	powder and pasteurized milk	[68]
AFB1	Electrochemical Aptamer-Based Method	2–500 nM	2 nM	89–112%.	wine	[69]
AFB1	Electrochemical Aptasensor	0.001–200 ng/mL	0.00033 ng/ mL	88.5–110.2%	peanuts and corn	[70]
AFB1	Electrochemical Aptasensor	0.125–16 ng/mL	0.12 ng/mL	92–102%	alcoholic beverages	[71]
AFB1	Electrochemical Aptasensor	5.0–750.0 pM	1.4 pM	99.37–102.2%	pistachio	[72]
AFB1	Electrochemical Aptasensor	2 nM–4 μM	2 nM	–	beer and white wine	[33]
AFM1	Electrochemical Aptasensor	3–90 ng/L	1 ng/L	–	milk	[73]
AFM1	Electrochemical Aptasensor	20–200 ng/L	5 ng/L	80 and 85%	milk	[74]
AFB1	Electrochemical Aptasensor	2.5–30 ng/L	1.6 ng/L	88.2–93.3%	coffee sample	[75]
AFB1	Electrochemical Aptasensor	0.1–10 ng/mL	0.086 ng/mL	80–96%	Maize flour	[76]
AFB1	Electrochemical Aptasensor	5 pg/mL–50 ng/mL	4.5 pg/mL	90.0–93.6%	peanuts	[77]
AFB1	Photoelectrochemical (PEC) Aptasensor	0.03–200 ng/mL	0.01 ng/mL	89.6–96.3%	peanut	[78]
AFB1	Photoelectrochemical (PEC) Aptasensor	0.01 ng/mL–100 ng/mL	0.002 ng/mL	98.3–106.7% 97.2–110.0%	peanut wheat	[79]
AFM1	Label-Free Aptasensor	10–500 fM	4.53 fM	–	pasteurized milk	[80]
AFB1	Electrochemical Aptasensor	0.1 pg/mL–10 ng/mL	0.049 pg/mL	94.5–106.7%	peanut oil	[81]
AFM1	Electrochemical Aptasensors	15–120 ng/L	8.47 ng/L	78.04–106.25%	milk	[82]

(*continued*)

Table 3. Continued.

Analyte	Sensor	Linear Range	LOD	Recovery	Sample Application	Ref.
AFB1	Electrochemical Aptasensors	0.5 nM–4μM	0.07 nM	94.58–104%	human blood plasma Pasteurized cow milk	[83]
AFB1	Electrochemical Aptasensors	10^{-6} −1 ng/mL	6.7×10^{-7} ng/mL	96% −103%	beer	[84]
AFM1	Electrochemical Aptasensor	2–600 ng/L	0.9 ng/L	91.3–96.5%	milk and serum s	[85]
AFB1	Electrochemical Aptasensor	0.01–1.0 fg/mL	0.002 fg/mL	96.8–106.0%	wine	[86]
AFM1	Electrochemical Aptasensor	5–120 ng/L	0.5 ng/L	–	whole cow or sheep milk	[87]
AFM1	Electrochemiluminescence Aptasensor	5–150 ng/mL	0.05 ng/mL	98.0–102.6%	milk	[88]
AFM1	Electrochemiluminescent Aptasensor	0.4 pg/mL–400 ng/mL	0.09 pg/mL	96.3–103.8%	milk	[89]
AFB1	Electrochemical Aptasensor	20 pg/mL–50 ng/mL	15 pg/mL	96.0–108.0%	peanut s	[90]

substances such as toxins, drug molecules, biomarkers, enzymes, nucleic acids, etc. The polymeric matrix is generated in the presence of the target molecule, and a three-dimensional network is obtained with strong interaction. MIP-based sensors show excellent selectivity due to providing the size, shape, and functional groups of the target (analyte) molecules.[134] After forming a polymeric matrix, the target molecules should be removed, and the cavities specific to the target should be obtained. The target should be rebinding the proper cavities for the re-recognition of the analyte molecules.[135] MIP techniques show excellent selectivity toward the target molecule and high specificity. Table 5 summarizes the analysis of aflatoxins in MIP-based electrochemical sensors.

Singh et al.[134] developed an electrochemical MIP using aniline as a monomer and AFB1 and FuB1toxins as template molecules (Figure 10). The obtained MIP-based biosensor exhibits a good LDR 1 pg/mL to 500 ng/mL. LOD 0.313 and 0.322 pg/mL for AFB1 and FuB1, respectively. Both MIP-based biosensors also exhibit satisfactory reproducibility, selectivity, and stability. Moreover, the MIP-based biosensor detects AFB1 and FuB1toxins in corn samples.

Optical biosensors for determination of aflatoxins

Optical methods based-biosensors constitute an important part of biosensor applications for the analysis of numerous analytes thanks to their significant features such as rapid, versatile, and label-free analysis, low cost, a portability, and real-time measurements. Additionally, it is possible to integrate optical biosensors with other multidisciplinary methods.[139,140] The measurement principle of optical biosensors is based on the emitted optical signal directly proportional to the concentration of the target analyte. For the signal measurement, optical biosensors can utilize different techniques: fluorescence, luminescence, surface plasmon resonance, refractive index, light scattering, colorimetric, etc.[139] In this context, optical biosensors are widely preferred for the analysis of aflatoxins.[141] It can be seen in Table 6, which summarizes the latest applications of optical biosensors for the determination of aflatoxins that the colorimetric assays and fluorescent biosensors have found a wide place in the literature in recent years due to the quick response time and accurate results.

In their study, Setlem et al.[142] focused on the determination of aflatoxin B1 (AFB1). Unlike chromatographic and immunological methods with disadvantages such as being expensive and time-consuming for AFB1 analysis, a selective, sensitive, and affordable method was developed in this study using an aptamer-based fluorescent sensor. Additionally, graphene oxide, which has great physicochemical properties, was used to provide adsorption of aptamers. The developed sensor showed a LDR 0.2 and 200 ppb. The accuracy and applicability of the sensor were demonstrated using groundnut, wheat, and fungal isolate samples. It showed high specificity and stability for the analysis of AFB1.

According to European union, maximum level of aflatoxin B1 and total aflatoxins in the dried fruits for desired for human intake are 2 μg/kg and 4 μg/kg, respectively while maximum levels of aflatoxin B1 and total aflatoxins (5 μg/kg, 10 μg/kg, respectively) in human consumption. To ensure that the produced food does not exceed this limit value, regular analyses are necessary. Low-detection-limits up to sub-picomolar and sub-femtomolar levels and wide linear analytical ranges were achieved with nanomaterials and nanocomposites of synergetic combinations. The comparison of aptamers-based biosensors, antibodies-based biosensors, and MIP-based biosensors by associating with LOD and LDR were given in Tables 3–5. When the recent applications of electrochemical aptasensors for aflatoxins were examined, it was observed that the LOD value of AFB1, OTA and AFM1 reached up to agmL^{-1}. Moreover, some of electrochemical aptasensors developed with copper nanoparticles (CuNPs) and gold nanoflowers modified screen-printed carbon electrodes can determine AFB1 in the range of 100 aM to 100 pM.[52] In another study, the proposed aptasensor based on the carbon quantum dots/octahedral Cu_2O nanocomposite was applied to measure aflatoxin B1. Taguchi method was used for providing the minimum number of experiments. A LDR of 3 agmL^{-1} −1.9 μgmL^{-1} and a LOD of 0.9 ± 0.04 agmL^{-1} were found.[60] A novel reduced graphene oxide/molybdenum disulfide/polyaniline@gold nanoparticles-based electrochemical aptasensor exhibits a wide LDR from 0.01 fg mL^{-1} to 1.0 fg mL^{-1} and a remarkably LOD of 0.002 fg mL^{-1} in spiked wine sample.[86]

When the recent applications of electrochemical immunosensor for aflatoxins were examined, it was observed that the LOD value of AFB1, AFM1 and AFM2 reached up to fgmL^{-1}. A metal free nanostructured g-C3N4 used for fabrication of biosensing platform was developed. The obtained electrochemical results indicate that the fabricated

Table 4. The summary of the analysis of aflatoxins in antibodies-based biosensors.

Analyte	Sensor	Linear Range	LOD	Recovery	Sample Application	Ref.
AFB1	Electrochemical Immunosensor	1.0 pg/mL–300 ng/mL	0.295 pg/mL	108.2%	fresh sweet corn	[91]
AFM1	Electrochemical Immunosensor	0.25–5.0 ng/mL	0.09 ng/mL	82.0–108.0%	milk	[92]
AFB1	Electrochemical Immunosensor	1.0 pM–5.0 nM	0.27 pM	90.38–99.64%	pu'er tea and rice	[93]
AFB1	Electrochemical Immunosensor	1.0 pg/mL–100 ng/mL	0.33 pg/mL	92.6–103.1%	peanut	[94]
AFB1	Sandwich-Type electrochemical Immunosensor	0.01–1.00 pg/mL	2.00 fg/mL	~100%	wheat	[95]
AFB1	Electrochemical Immunosensor	1 fg/mL–1 ng/mL	0.328 fg/mL	–	–	[96]
AFB1	Label-Free Electrochemical Immunosensor	0.001–10000 ng/mL	0.00054 ng/mL	95.6–98.6%	corn extract	[97]
AFB1	Electrochemical Immunosensor	0.1–25 nM	0.1 nM	95–100%	spiked maize	[9]
AFB1	Electrochemical Catalase Sensor	0.5–3.5 U/mL	0.434 U/mL	–	*Aspergillus flavus* extract	[98]
AFB1	Electrochemical Biosensor	0.5–100 ng/mL	0.15 ng/mL	80.74–111.22%	milk	[99]
AFB1	Electrochemical Immunosensor	0.05–20 ng/ mL	0.033 ng/mL	–	rice, wheat, corn, sorghum, barley, buckwheat	[100]
AFB1	Quantum Dot–Based Electrochemical Immunosensor	0.08–800 μg/kg	0.05 μg/kg	83.9–118.0%,	peanut oil wheat maize husky rice	[101]
AFB1	Electrochemical Immunosensor	0.5–10 ng/mL	0.5 ng/mL	–	pistachio matrices	[102]
AFB1	Electrochemical Immunosensor	0.01 − 1 ng/mL–25 ng/mL	0.001 ng/mL	–	vegetable oil	[103]
mycotoxin	Electrochemical Immunosensor	0.1–3.0 ng/mL	0.09 ng/mL	80.2–98.3%	spiked maize	[104]
AFB1	Photoelectrochemical Sensor	0.005–50 ng/mL	0.0015 ng mL	96.7–103.3%	wheat and peanut samples	[37]
AFB1	Disposable Paper-Based Immunosensor	1–30 ng/mL	0.62 ng/mL	97–99%,	maize flour	[105]
AFB1	Electrochemical Enzyme-Linked Immunosorbent Assay	0–120 ng/mL	0.15 ng/mL	93.37–104.01%	milk	[106]
AFB1	Electrochemical Competitive Immunosensor	0.5–10 ng/ mL	68 fg/mL	86.44–107.20%,	corn	[107]
AFB1	*Escherichia Coli*-Based	0.01–0.3 μg/mL	1 ng/mL	94.4–101.3%	peanut and corn oils	[108]
ZEN	Electrochemical Biosensor	0.05–0.5 μg/mL	6 ng/mL	102.4–106.0%		
AFB1	Electrochemiluminescence Immunosensor	0.05–100 ng/mL	0.01 ng/mL	99.20–100.97%	lotus seed	[109]
AFB1	Electrochemical Immunosensor	0.05–25 ng/mL	0.05 ng/mL	98.9–101.2%	rice	[110]
AFB1	Electrochemical Immunosensor	0.025–30 ng/ mL	0.006 ng/mL	–	peanut	[111]
AFB1	Electrochemiluminescence Sensor	0.1–100 ng/ mL	0.033 ng/mL	86.4–123%	grains	[112]
AFB1	Electrochemical Immunosensor	0.025–60.00 ng/mL	0.0052 ng/mL	103.6–104.1%	rice and peanut	[113]
AFB1	Electrochemical Immunosensor	0.025–25.00 ng/mL	0.017 ng/mL	98.0–101.1 %	rice	[114]
AFB1	Electrochemical Immunosensor	0.01–10 ng/mL	0.001 ng/mL	–	peanuts	[115]
AFM1	Electrochemical Immunosensor	0.01–1 μg/L	0.009 μg/L	96.15–104.25%	milk	[116]
AFB1	Label-Free Immunosensor	50 fg/mL–5 ng/mL	50 fg/mL	–	rice milk	[117]
AFB1	Electrochemical Immunosensor	$10^{-3} - 2 \times 10^{4}$ ng/mL	0.159 pg/mL	93.5–102.3%	rice, peanuts and corn	[118]
AFM1	Electrochemical Immunosensor	0.01–1 μg/L	0.02 μg/L	–	milk	[119]
AFB1	Photoelectrochemical Immunoassay	0.01–15 ng/mL	2.8 pg/mL	90.2–109.0%	peanut samples and corn	[120]
AFB1	Electrochemical Immunosensor	0.1–3.0 ng/mL	0.008 ng/mL	–	maize	[121]
AFM1	Electrochemical Immunosensor	0.010–1 μg/L	0.010 μg/L	–	milk	[122]
AFB1	Photoelectrochemical Immunoassay	0.01–10 $ngmL^{-1}$	2.6 pg/mL	–	peanut	[123]
AFB1	Electrochemical Immunosensor	100 ng/mL −1 pg/mL	6.9 pg/mL	97.6–102.8%	peanut	[124]
AFB1		0.01–10 ng/mL	0.01 ng/mL	94.0–130%	powered milk beer	[125]

(*continued*)

Table 4. Continued.

Analyte	Sensor	Linear Range	LOD	Recovery	Sample Application	Ref.
	Label-Free Impedimetric Immunosensors					
AFB1	Electrochemical Immunosensor	0.01–100 ng/mL	3.3 pg/mL	93.27– 106.70%	wheat	[126]
AFB2	Electrochemical Immunosensor	0.1 pg/mL–100 ng/mL	0.10 pg/mL	95.84–106.3%	corn	[127]
AFM1	Electrochemical Sensor	0.015–25mM	25mM	98.10–112.0%	milk	[128]
AFB1	Electrochemiluminescence (ECL) Immunosensor	0.01–100 ng/mL	0.0039 ng/mL	95%–101%	fresh milk	[129]
AFB1	Electrochemical Immunosensor	0.1–100 ng/mL	0.05 ng/mL	98.5–106.3%	corn	[130]
AFB1	Electrochemical Immunosensor	0.0001–10 ng/mL	0.3 pg/mL	80–127%.	peanut	[131]
AFB1	Electrochemical Immunosensor	0.5–20 ng/mL 20–60 ng/mL	0.109 ng/mL	92.18–95.4%	maize	[132]
AFB1	Label-Free Electrochemical Immunosensor	0.05–25 ng/mL	0.027 ng/mL	85.94–111.60%,	wheat	[133]

Table 5. The summary of the monitoring of aflatoxins in MIP-based biosensors.

Analyte	Sensor	Linear Range	LOD	Recovery	Sample Application	Ref.
AFB1	MIP Based Biosensor	50.0 pg/L–3.5 ng/ L 3.5–40.0 ng/L	12.0 pg/L	97–104%	milk	[135]
AFB1	MIP Based Biosensor	3.2 fM and 3.2 μM	3.0 fM	–	–	[136]
AFB1 Fumonisin B1 (FuB1)	MIP Based Biosensor	1 pg/mL–500 ngmL	0.313 pg/mL 0.322 pg/mL	91.62–108.61%	corn	[134]
AFB1	MIP Based Biosensor	0.10 ng/mL–10 ng/mL	0.058 ng/mL	93.5–112.8%	rice and peanut	[137]
AFB2	Electrochemical Imprinted Sensor	0.1–1000 fg/mL	0.2 fg/mL	90–99%	dairy milk	[138]

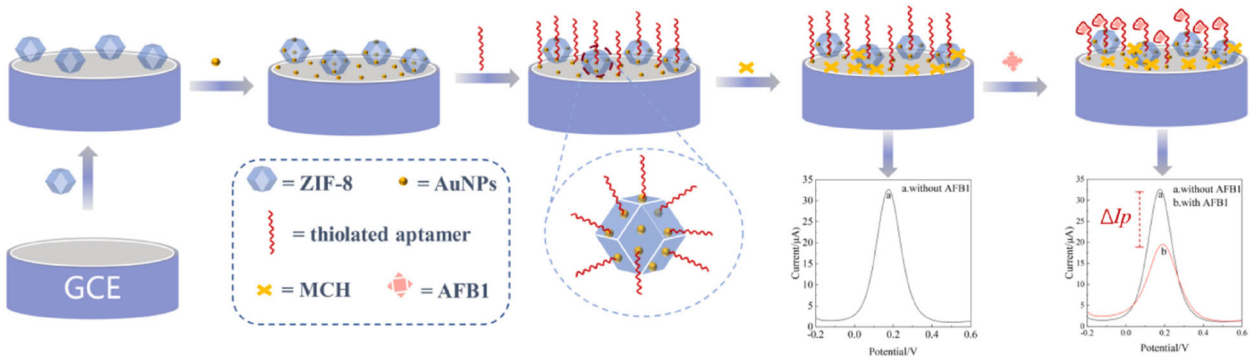

Figure 8. Schematic of the designed electrochemical aptasensor for AFB1 detection. Reproduced with permission from Elsevier.[45]

biosensing electrode having ability to detect AfB1 with lower LOD of 0.328 fg mL^{-1}, LDR in between 1 fg mL^{-1} to 1 ng mL^{-1}.[96] Ag nanocubes incorporated trigonal metallic MoS2 nanosheets with 1 T phase as signal amplification and gold nanoparticles/porous graphene nanoribbon as an electrochemical sensor platform were used for development of a novel electrochemical AFB1 sandwich immunosensor. The fabricated biosensor system detected AFB1 with lower LOD of 2.00 fg mL^{-1}, LDR in between 0.01–1.00 pg mL^{-1}.[95]

When the recent applications of electrochemical molecularly-imprinted sensor for aflatoxins were examined, it was observed that the LOD value of AFB1, FuB1 and AFB2 reached up to $pgmL^{-1}$. Jiang M. reports that a sensitive electrochemical molecularly-imprinted sensor was developed for the detection of AFB1, by electropolymerization of p-aminothiophenol-functionalized gold nanoparticles in the presence of AFB1 as a template molecule. The molecularly-imprinted sensor exhibits a broad LDR in between 3.2 fM and 3.2 μM, and a LOD of 3 fM.[136]

Quartz crystal microbalance (QCM) techniques for determination of aflatoxins

Quartz crystal microbalance technique (QCM) is a mass-sensitive transducer device that has advantages such as high sensitivity, real-time output and, low cost. This device provides measurement by considering the dependence of the oscillation frequency on the total mass of the oscillating transducer and detecting the change in frequency of a quartz crystal resonator.[155,156] The QCM-based immunosensor has been the focus of research into biomolecular interactions.[156] Recently, QCM-based sensors have emerged as a potential way to investigate interactions on molecular basis solid surfaces and the quantitative analysis of various

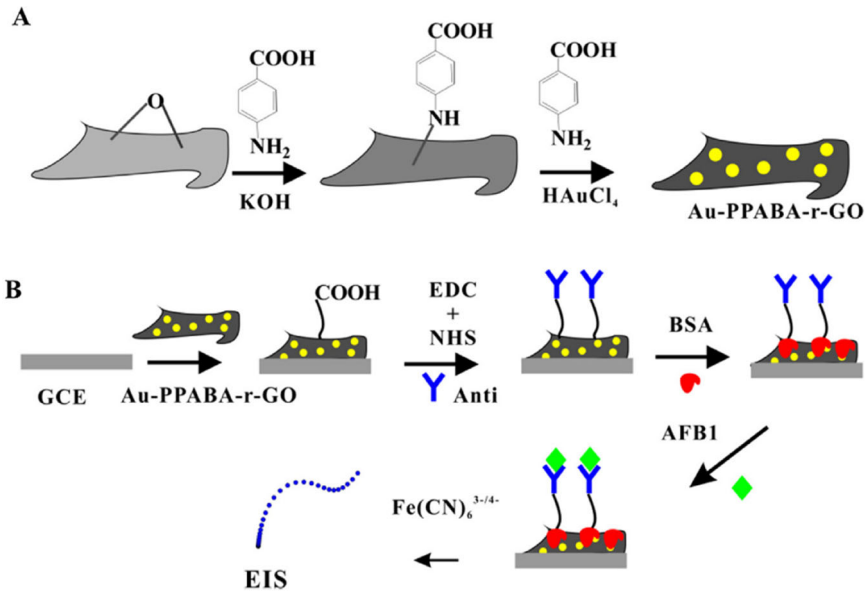

Figure 9. Schematic of the designed electrochemical immunosensor for AFB1 detection. Reproduced with permission from Elsevier.[103]

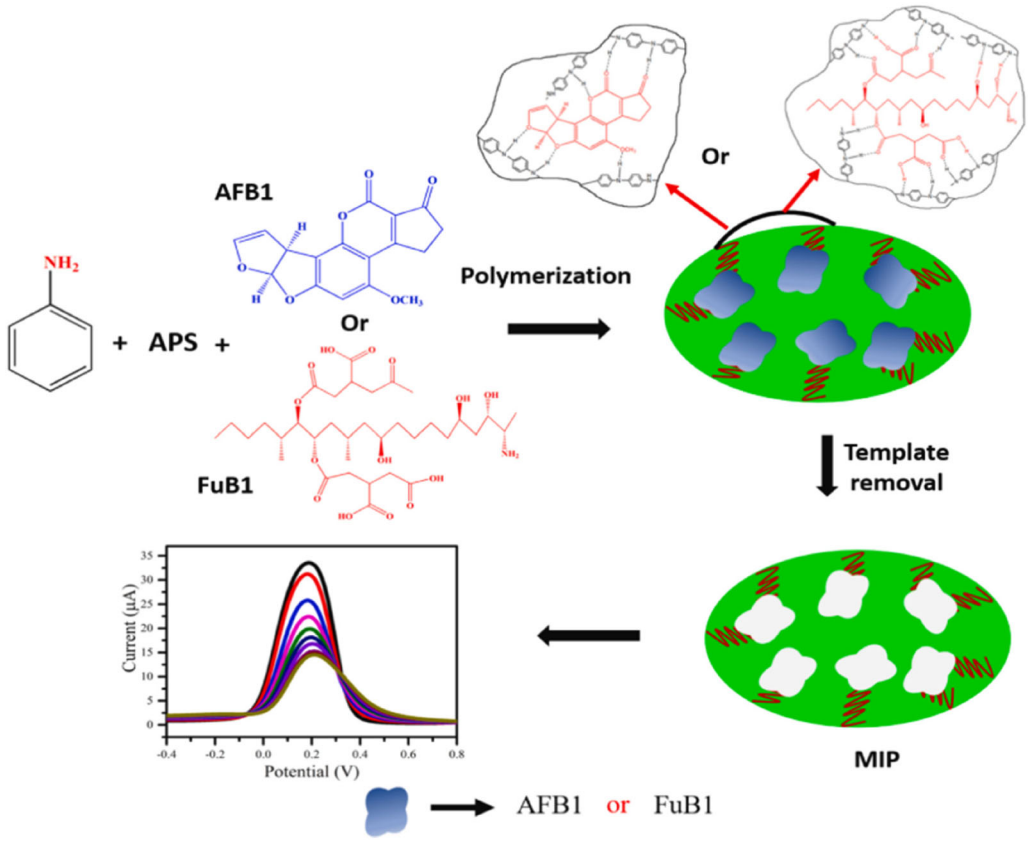

Figure 10. Schematic of MIP-based sensing process. Reproduced with permission from Elsevier.[134]

analytes.[157–159] Molecularly imprinted polymers (MIPs) are crosslinked polymers containing recognition sites that are complementary to the templates in shape and size.[160,161] MIP has attracted popularity in biomedical field environmental and food quality analysis because of rapidness, affordability, selectivity, high sensitivity, stability, and low analyte requirement.[162] The use of QCM and MIP together has the potential to provide an excellent sensing platform.[163] Gu et al.,[156] were created for the first time a quartz crystal microbalance (QCM) sensor for the detection of aflatoxin B1 using a gold nanoparticle (AuNPs) doped molecular imprint layer and covalent organic frameworks (COFs-AuNPs). The QCM detection platform for the measurement of AFB1 was obtained by electropolymerization of o-ATP@AuNPs crosslinked MIP on the COFs-AuNPs modified electrode. The composite of COFs-AuNPs and the

Table 6. Latest applications of optical biosensors for determination of aflatoxins.

Analyte	Sensor	Linear Range	LOD	Sample Application	Ref.
AFB1	Fluorescent aptasensor	0.2–200 ppb	20 ppb	Groundnut, wheat, fungal isolate	[142]
AFB1	Microfluidic paper-based analytical device-colorimetric assay	8–25 ng.mL^{-1}	9.45 ng.mL^{-1}	Corn, peanut, pasta, baijiu, soy sauce, eight-treasure porridge	[143]
AFB1	Colorimetric nanosensor	NA	1.47 ng.g^{-1}	Packaged peanut and corn	[144]
AFB1	Fluorescence sensor	0.05–100 ng.mL^{-1}	0.01 ng.mL^{-1}	Corn, wine	[145]
AFB1	LSPR	0.1–500 nM	0.4 nM	Maize	[9]
AFB1	LSPR	0.2–6 ng.mL^{-1}	0.09 ng.mL^{-1}	Peanut, animal feed	[146]
AFB1	Colorimetric immunoassay	1–75 ng.mL^{-1}	0.16 ng.mL^{-1}	Nut, cornflake, cornmeal, peanut, peanut butter, pecan nut	[28]
AFM1	Colorimetric aptasensor	30–75000 ng.L^{-1}	30 ng.L^{-1}	Milk	[147]
AFB1	Colorimetric enzyme-linked immunosorbent assay	0.06–0.48 μg.L^{-1}	0.06 μg.L^{-1}	Peanut, maize	[148]
AFB1	Fluorescence sensor	1 pg.mL^{-1}–100 ng.mL^{-1}	0.98 pg.mL^{-1}	Peanut, corn, wheat, Chinese medicine samples	[149]
AFM1	Fluorescent aptasensor	0.0001–0.5 ng.mL^{-1}	0.194 pg.mL^{-1}	Milk	[150]
AFB1 AFB2	Fluorescence spectroscopy	2.7–320.2 ng.g^{-1}	0.2 ng.g^{-1}	Almond	[151]
AFB1	Colorimetric sensor	0–5 mg.mL^{-1}	0.22 mg.kg^{-1}	Rice	[152]
AFB1	Fluorescent aptasensor	0.1–10 ng.mL^{-1}	21.3 pg.mL^{-1}	Corn flour	[153]
AFB1	SPR	0.0001–10 ng.mL^{-1}	1.04 pg.mL^{-1}	Peanut, corn	[154]

AFB1: Aflatoxin B1, NA: Not available, LSPR: Localized Surface Plasmon Resonance, AFM1: Aflatoxin M1, SPR: Surface Plasmon Resonance.

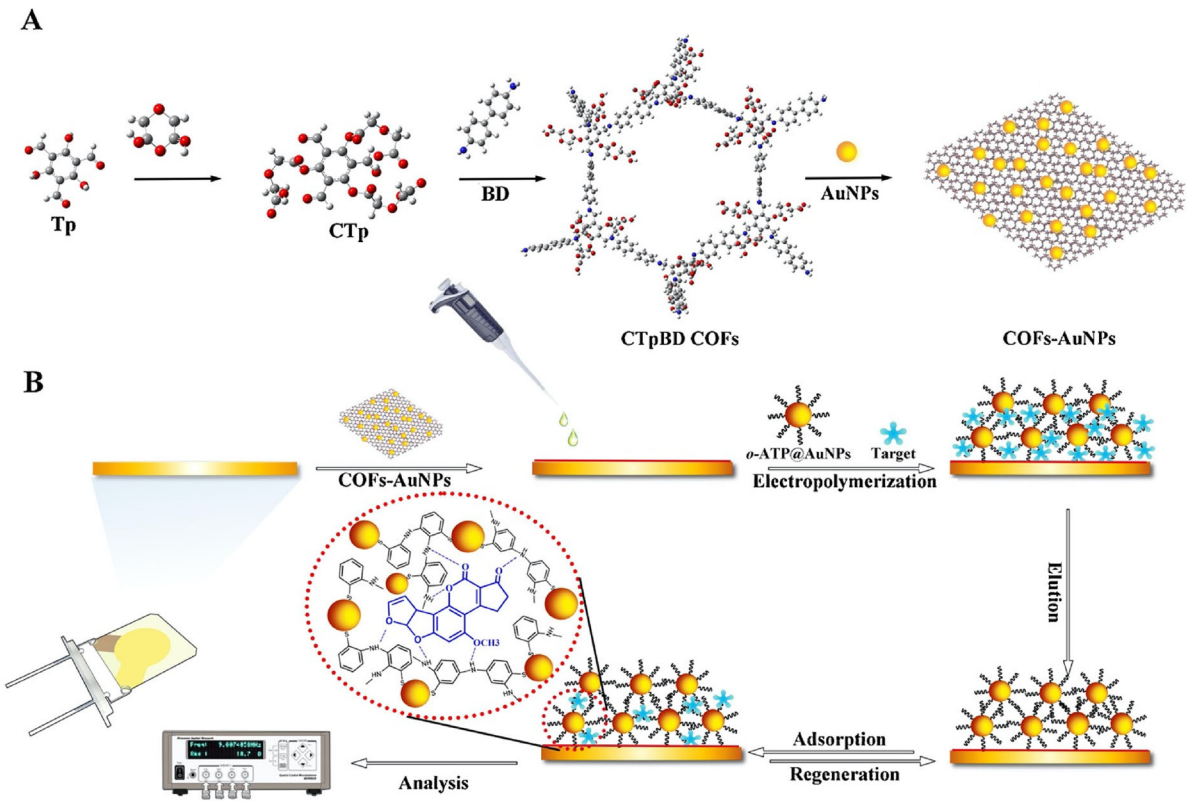

Figure 11. (A) Demonstration of the fabrication process of nanomaterials, (B) the preparation and analysis of fabricated QCM sensor. Reproduced with permission from Elsevier.[156]

imprinted polymer of AuNPs with poly(o-ATP) were used to achieve sensitive and selective detection. Specific recognition sites incorporated into the o-ATP@AuNPs crosslinked MIP matched to identify AFB1, and the COFs-AuNPs composite material created high specific surface area (Figure 11). The AuNPs doped imprinted matrix was endowed with additional recognition sites thanks to the three-dimensional structure of o-ATP@AuNPs crosslinked MIP and the

synthesized COFs-AuNPs substrate. The sensor was also used to analyze real samples (peanuts, pistachios, rice, and wheat). In real samples analysis, the suggested technique had LDR 0.05 to 75 ng mL^{-1}, a low LOD 2.8 pg mL^{-1}), and excellent recoveries (87.0–101.7%). This research presents a QCM sensing platform that is simple, resilient, low-cost, sensitive, and selective, and has a lot of real-world promise. . In another work, a QCM detection platform for acrylamide (AM) detection was successfully implemented by electropolymerized the 3-thiopheneacetic acid functionalized AuNPs (3-TAA@AuNPs) crosslinked print layer on nitrogen-doped ordered mesoporous carbon modified-AuNPs (NOMC-Au) modified layer by Chi and Liu. The NOMC-Au-modified layer had a large specific surface area, which gave more recognition space to the cross-linked printed layer to significantly improve the detection performance. By using 3-TAA@AuNPs as a functional monomer and PM as a fictitious template molecule, the crosslinked imprinted layer was able to pair well with AM as it had more crosslinked units. The final QCM detection platform (MIP/NOMC-Au/AuE) exhibited a good linear range for AM from 0.08 to 100 ng/mL. The recovery rate of the three additional concentrations of the three samples was between 88.3% and 97.2%, with a limit of detection (LOD) of 5.1 pg/mL. The QCM sensor's preparation process was also very controllable, making it possible to construct sensing platforms for the examination of food samples that meet various requirements.[164] Finally, based on bridging interactions between Au-electrode and NH_2-TiO_2 sites enriched with phosphate groups, a new QCM sensor for ultrafast detection of trace phosphoprotein was developed by Guo et al. Here, NH_2-TiO_2 was immobilized using 11-mercaptodecanoic acid (MUA) modified with an Au-S bond. NH_2-TiO_2 with phosphoprotein absorption functionality. With a low detection limit of 5.3 106 mg mL^{-1}, the proposed sensor exhibited linearity depending on the casein concentration between 1.0103 and 1.0 mg mL^{-1} under ideal conditions. The limit of quantitation was found to be 0.001 mg mL^{-1}. The analysis method of the developed NH_2-TiO_2/MUA/AuE-QCM sensor was less complex and faster than the traditional Ti^{4+}-IMAC/MOAC system and could be completed in less than 5 minutes. Additionally, the created biosensor was effectively applied to the detection of chicken egg white and skim milk.[165]

Conclusion

Here, recent advances in the electrochemical and optic biosensing and QCM technique were illustrated for the determination of aflatoxins. Most of these studies focused on the Aflatoxin B1, B2, G1, and G2 due to their high toxicities and high incidence. For the determination of aflatoxins, biosensor applications have been a popular topic recently. This review explains the novel and the most recent biosensor approaches and applications for the determination of aflatoxins in clinical and non-clinical (food and beverages) samples. Electrochemical biosensors (aptamers-based biosensors, antibodies-based biosensors, MIP-based biosensors), optical biosensors, and QCM techniques have displayed comparable accuracy and reliability to traditional methods.

The nanomaterial based optical and electrochemical sensors have a bright and exciting future. The aim of future research is to improve the detection methods of nanomaterial technology, expand the detection range and simplify the fabrication of sensor. Moreover, microfluidic systems should be improved for food toxin from various food products and agriculture produces. In this review, many aptamers-based biosensors were observed for determination of Aflatoxins, and they have the potential to become commercially available in the future.

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