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Recent Developed Ultrasensitive Methods for Ochratoxin a Detection Using Fluorescence Analyzer Devices and Aptasensors

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Abstract

Mycotoxin are secondary metabolite of molds which can cause acute or chronic toxicological effects. One type of mold can produce different types of mycotoxin and its production is affected by various factors. Molds are easily to grow on food product like cereals, coffee, beans, nuts, vegetables and fruits. Also, mycotoxins are founded in commercialized processed product like bread, wine, milk, other milk product, beer, chocolate products and even in meet and meet product because during food processing mycotoxin cannot be eliminated. Especially, OTA is the most mycotoxin with the nephrotoxic, immunotoxic, teratogenic and carcinogenic effects. The International Agency for Research on Cancer (IARC) has classified OTA in 2B Group (possibly carcinogenic agent) The best way to protect consumers against mycotoxin effect is early food detection. In recent years, various methods have been introduced for detection of OTA. However, they are usually time-consuming, labor-intensive and expensive. Therefore, these parameters limited their usage. The emerging method of detection, aptasensor, has attracted more attention for OTA detection, due to distinctive advantages including high sensitivity, selectivity and simplicity. In this review, the new developed Fluorescence analyzer optical devices and aptasensors for detection of OTA have been investigated. We also highlighted advantages and disadvantages of different types of OTA detection methods. This review also takes into consideration the goal to find out which designed methods are the most rational ones for highly sensitive detection of OTA.

Keywords

Ochratoxin A, Aptasensors, Detection, Fluorescence Analysis, Colorimetrical, Electrochemical, Electrochemical

1. Introduction

Moulds are not foodborne pathogens by themselves, they can produce an array of secondary metabolites (mycotoxins) with acute or chronic toxicological effects. Fungi are a large group of diverse eukaryotic organisms which include yeasts and moulds. Moulds (filamentous fungi) are widely distributed in nature. Due to their versatile nutritional requirements, they are common contaminants and under favorable conditions of humidity and temperature, propagate on different commodities and beverages and produce mycotoxins. Nevertheless, mycotoxins, as natural contaminants are not easy to control for both producers and exporters [1], [2]. Mycotoxins contamination represents a worldwide problem in terms of human/animal health and furthermore can pose a heavy economic burden to the industry. Mycotoxins can contaminate a product all over the food chain, in the field as well as during storage, or at later points [3]. Contamination of human food and livestock feed by fungi and their respective toxins presents a serious food safety issue globally, leading to incredible yield and economic losses [4].

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production is affected by various factors. Molds are easily to grow on food product like cereals, coffee, beans, nuts, vegetables and fruits. Also, mycotoxins are founded in commercialized processed product like bread, wine, milk, other milk product, beer, chocolate products and even in meet and meet product because during food processing mycotoxin cannot be eliminated [1].

Ochratoxin A (OTA) a mycotoxin produced by *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Penicillium verrucosum* genera under different environmental conditions. OTA is one of the most the most food contaminating mycotoxin. Human can be contaminated through consumption of contaminated food products, such as contaminated grain, pork and coffee product as well as wine and dried grapes. OTA has been found in the tissues and organs of animals, including human blood and breast milk [5], [6]. OTA is one of the most commonly encountered mycotoxins, and was reportedly detected in 60% of a healthy Moroccan study population of blood donors. OTA has been hypothesized to cause oxidative damage to DNA, leading to mutagenesis and potential carcinogenesis. Recent papers also propose direct genotoxic mechanisms for OTA, describing a pathway that metabolizes OTA into an electrophilic species capable of directly binding to some nucleotide bases [7].

Several studies have introduced OTA as a nephrotoxic, hepatotoxic, neurotoxic, immunotoxic, and also teratogenic agent. Lymphoid and gastro-intestinal tract lesions, myelotoxicity, and intestinal fragility have been reported as other toxic effects of OTA in animal tissues investigation. In immune system, not only OTA inhibits T and B lymphocytes proliferation, but also abolishes IL2 production. Also, it is responsible for human Balkan endemic nephropathy (BEN) and urinary tract tumors [8]. Consequently, OTA is classified as a possible human carcinogen by IARC, citing sufficient evidence of carcinogenicity in animal models, but insufficient evidence from human studies. A recent in vivo study using mice reported that p53 tumour suppressor protein was upregulated during OTA treatment, and investigated the extent to which the p53 protein inhibits progression of OTA-induced DNA damage. OTA is known to bind to blood plasma proteins, delaying its excretion in urine by up to 30 days, a fact greatly relevant to the development of methods for exposure detection. Despite this temporary bioaccumulation, no significant relationship was found between age and plasma OTA levels in a British study population [1, 9-12]. European Commission has established regulatory limits for raw cereal grains (5 μg/kg), dried fruits (10 μg/kg), roasted coffee (5 μg/kg), grape juice and all types of wines (2 μg/kg) [13, 14]. To protect consumers, it is of great significance to develop sensitive, cheap, fast and easy to use OTA detection methods and techniques for both small and large-scale operations at key supply chain points for instance by grain handlers and agricultural product manufacturers. Thus, it is of supreme importance to have an appropriate analytical technique for the high sensitive detection of OTA. Several methods have been developed to detect OTA including mass spectroscopy, high performance, and antibody-based techniques [15]. In general, these methods are time-consuming, labor intensive and require high-tech equipment and trained operators [16]. Hence, it is of particular interest to develop and employ a sensitive, accurate, rapid, and easy-to-operate method for OTA detection. The objective of present review paper is to make revision on recently cheap, portable and field effective tools based on fluorescence analysis for OTA. In addition, we focused on aptamers as recognition elements for OTA sensing due to their unique properties with their application as recognition elements provides sensitive aptamer-based sensors (aptasensors).

2. Fluorescence Analysis Method

Optical detection is one of the oldest and most established techniques [17] and has emerged as a method of choice by its simple assembly in the miniaturization of instruments for field measurements, involves the use of light emitting diode (LED) as emitter or detector. LED based systems have already been explored for sensing of different analytes (hemoglobin, O_2 and available phosphorus in soil) [18]. The advantages of this instrument are: functional simplicity, low-cost, low power consumption, high stability, efficiency of light production within a narrow wavelengths band, longevity [19] and market availability with diverse wavelengths [20], [19]. It is easy to acquire LEDs that substitute the complex optical system obtained, when monochromatic LEDs are used as source of several wavelengths in the visible region [20]. Considering the use of the optical methods [20], [21] with LED-based instruments has emerged as an alternative in the determination of many chemical parameters. OTA exhibits natural fluorescence at certain wavelengths, hence it becomes easy to develop sensitive detection systems using fluorescence techniques. Moreover, fluorescence has been the most commonly used form of detection in microscale biological and chemical analysis, primarily due to high sensitivity, and capable of being miniaturized into credit card size microfluidic lab-on-a-chip (LOC) systems and small portable systems [22].

Considering the fluorescence and the colorimetric detection in a pixel of the samples, the smallest units from which an image is composed; the pixels given to us by the display device, are measurements of the light being emitted by the sample. From these data, we can make deductions, more light may indicate the presence of a particular structure or substance, and knowing the exact value allows us to make comparisons and quantitative measurements. The purpose of RGB color model is for the sensing, representation, and display of images in electronic systems, such as televisions or computers. This color model is a device dependent, different devices detect or reproduce a given RGB value differently and their response to the individual R, G, and B levels. The ratio of some RGB coordinates is used for calibration purposes using digital image processing [23]. A low cost and portable fluorescence detection device for the analysis of OTA was developed by exploiting the RGB model in

connection with the image sensors to design colorimetric assays for detection of various analytes or compounds qualitatively and quantitatively. The design incorporates a sensing module, which consist of an ultraviolet light emitting diode to excite the fluorescence of the OTA and the emission was registered with the CMOS image sensor of a serial port camera [24].

D. Bueno Hernández et al. (2017) successful developed a portable and low-cost fluorescence set-up to quantify the concentrations of Ochratoxin A (OTA) in real samples using a developed system. The detection through the set up consist of an ultraviolet light at 365 nm and a CMOS sensor controlled by an executable interface designed in MAT-LAB, with a time consumed from acquisition to processing to image display of 20 seconds. It is reported that OTA is naturally fluorescent, so it allows the user to get a photograph of the OTA under excitation conditions and process the image in order to predict the concentrations of the sample. To capture and process the image, in an automatically manner, the system was completely based on the Red, Green and Blue (RGB) components. For each concentration of the OTA, the R, G, B coordinates were obtained and plotted to quantify the mycotoxin presented in the sample. The linearity for OTA was obtained in the concentrations range of 5–40 µg/L. Immunoaffinity columns (IAC) and molecular imprinted polymer columns (MIP) were compared. The maximum recovery of 107.76% was recorded for 2 µg/L of OTA using IAC in beer samples. The obtained results were cross validated using chromatographic method HPLC. The developed setup is easy to use, economical and portable [24].

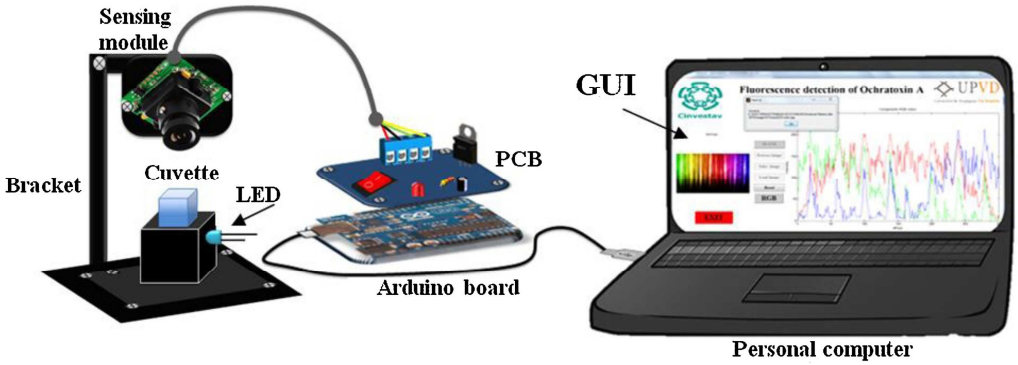

Figure 1. *Sensing module connected to the final device and its control with the computer [24].*

Bueno (2016) Develop a fluorescence analyzer for OTA using the smartphone camera and an UV light emitting diode (LED) to obtain an image of the fluorescence of OTA by wireless from the smartphone to the personal computer. The study describes an innovative approach to quantify the concentrations of ochratoxin A (OTA) using the smartphone as fluorescence device. The detection through the smartphone is based on the fluorescence phenomenon. It occurs when the sample is excited with ultraviolet (UV) light, the fluorescence from excited sample passes through a lens to smartphone camera. Finally, it is send by wireless to personal computer. The fluorescence image data from the smartphone camera is analyzed by a personal computer; then, the images are represented in its Red, Green and Blue (RGB) components. It is reported that OTA is naturally fluorescent, hence when the light is emitted through solution with OTA; it provides a blue fluorescence, so the image is captured with the smartphone camera whereas for blank solution, no fluorescence was observed. To process the image, a graphical user interface (GUI) in MATLAB R2011a was made. The best linear range with the developed system was in the range 2–20 µg/L and the limit of detection (LOD) was 2 µg/L. This is comparable with commercial equipment in a portable system and employing smartphone as a power source also [25].

Figure 2. *Fluorescence analyzer developed based on smartphone camera [25].*

3. Aptamer-Based OTA Biosensors

Aptamers are short single-stranded oligonucleotides, either RNA or DNA, that fold into well-defined 3-D structures and bind to their ligand by complementary shape interactions. They bind to their targets (e.g. small molecules, peptides and proteins) with high affinity and specificity, have a high inhibitory potential, are not toxic or immunogenic, and can be produced synthetically. They are identified by an *in vitro* selection procedure, also called SELEX (systemic evolution of ligands by exponential enrichment), that was recently fully automated [26]. Aptamers exhibit high affinity and selectivity for protein targets as well as antibodies. In addition, they provide certain features such as broad range of targets, high reproducibility, availability, easy production, and high stability [27], [28]. In recent years, various aptasensors have been introduced for the detection of OTA [29], [30] which functioned through various detection modes, including optical sensors such as electrochemiluminescent [31], [32], colorimetry [33], localized surface plasmon resonance (LSPR) [34], fluorescence [27] and electrochemical [35] aptasensors which are investigated, respectively.

3.1. Optical Aptasensors

Optical transduction have been employed in a large numbers of biosensors for detection of food toxins [36]. Generally, optical aptasensors consist of a transducer that can capture signals originated from a chemical, biological or physical phenomenon and turned them into ultraviolet (UV), visible or infrared (IR) radiations. Optical methods, including colorimetric, fluorescent, chemiluminescence and surface plasmon resonance, provide high sensitivity and benefit from simple and rapid operation. Thus, majority of developed biosensors rely on optical transductions.

3.1.1. Colorimetry-Based Aptasensors

Colorimetry methods are the most common types of optical sensors as they have simple sensing mode [37] Colorimetric analysis is a method of detection of a chemical compound in a solution with the aid of a color reagent [38]. In this method, detection can be done without requirement of costly and complicated tools [39]. In some colorimetric systems, color changes can be observed with the naked eyes. These features enable users to accurately detect targets without any access to sophisticated and expensive equipment.

Colorimetric sensors employ nanomaterials such as gold nanoparticles (AuNPs) function based on size-dependent optical properties of the employed nanomaterial. It has been shown that assembly/disassembly property of theses NPs is an important indicator in colorimtric assays. By using aggregation/dispersion behavior of AuNPs, the presence of a particular analyte can be monitored.

Based on analyte-induced assembly of oriented AuNPs dimmers, Xiao et al. (2015) demonstrated the first colorimetric aptasensor for sensitive detection of OTA. When OTA aptasensor is presented, with Y-shaped duplex, probe 1 and probe 2 are linked together. In addition, this structure plays as a linker to assemble two gold nanoparticles to form a dimmer. In the next step, when OTA is added to solution, it would compete with probe 1 and probe 2 for forming duplex with aptamer. Consequently, the asymmetric assembly will be destroyed. Finally, solution color would change from blue to red. Limit of detection (LOD) in this system was determined 0.05 nM. This method overcome limitations such as stability, sensitivity, and detection range which existed in the previous systems [40].

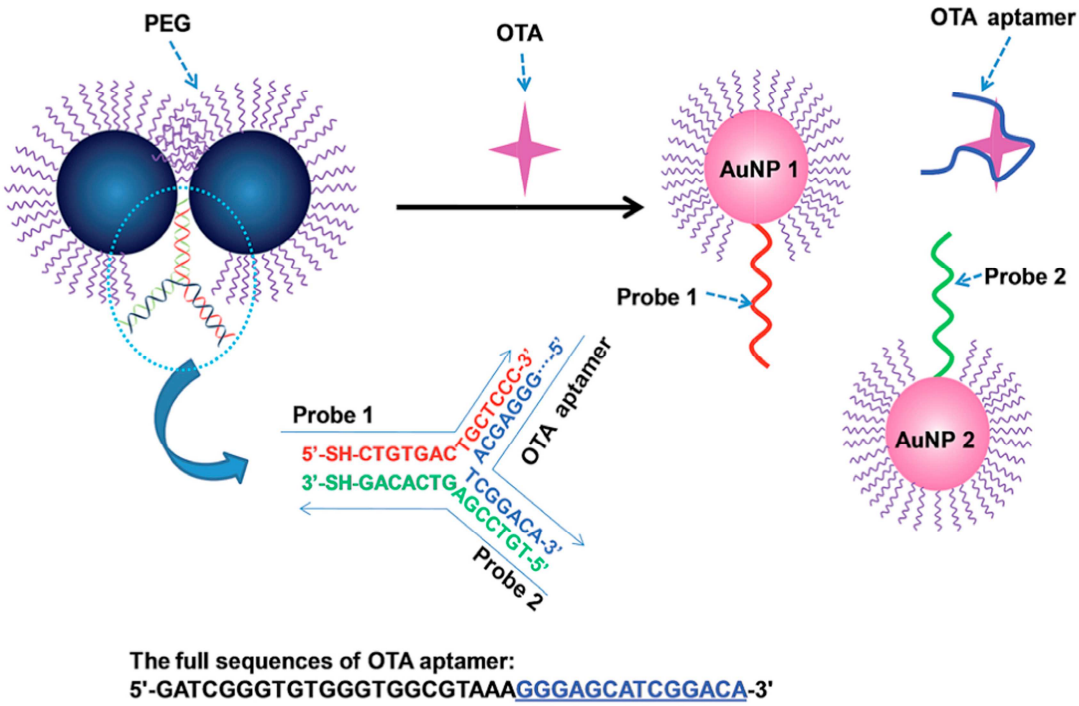

Figure 3. *Sensing mechanism of the AuNP dimer-based colorimetric aptasensor [40].*

3.1.2. Chemiluminescence-Based Aptasensors

Chemiluminescence (CL) signals can be strengthened by using chemiluminescence resonance energy transfer (CRET). CRET is a kind of non-radiative dipole-dipole transfer of energy from a donor molecule to an acceptor dye. In contrast with FRET, in this procedure, luminescent substrate can be specifically oxidized during chemical reaction with no need to have an external light source [41]. Employing CRET aptasensor, a novel sensitive, and non-competitive platform was developed for specific detection of OTA. 5′-DNAzyme-Linker-OTA aptamer-3′-dabacyl complex was used as aptamer sequence. Dabacyl functioned as a quencher in CRET aptasensing system. In the absence of OTA, hemin/G-quadruplex (HRPzyme) structure was formed upon addition of hemin to the system. In this state, higher chemiluminescence yield with low quenching could be achieved. OTA addition to the environment, resulted in the formation of second quadruplex system (OTA/G-quadruplex) which decreased chemiluminescence signal [41]. In the range of 0.1–100 ng mL−1, with increasing OTA concentration, signal would decrease. LOD of this model was 0.22 ng mL−1. Owing to its quick performance (30 min), highly specific, and simple sample preparation, this system has high potential to be used as an on-site screening instrument [41].

Employing CRET, Mun et al. [42] developed another method for determination of OTA using DNAzyme-linked aptamer. Single-stranded DNA (ssDNA) consists of aptamer, linker and DNAzyme. Quencher molecule was attached to the 3′-end of aptamer. In the presence of H_2O_2 and luminol, hemin was incorporated into the G-quadruplex of DNAzyme, resulted in CL. Once target molecules (i.e. OTA) were present in the solution, the sequence of aptamer was folded, owing to the complex formation between aptamer and the target molecule. Then, the quencher dye was closed to DNAzyme structure and CRET occurred. This platform was able to determine OTA with LOD of 0.27 ng/mL, with no need for the elimination of unbound aptamer or phase separation [42].

3.1.3. Surface Plasmon Resonance-Based Aptasensors

In general, surface plasmon resonance (SPR) sensors are categorized as biosensors that utilize the excitation of surface plasmons for sensing purposes [43]. In SPR biosensors, adsorption of target molecules on specific ligands immobilized on the surface of a chip, leads to change in refractive index. SPR approach is able to perform qualitative and quantitative analysis of interactions occurring among biomolecules such as proteins, small molecules, and oligosaccharides in real-time without labeling [43]. Improving the properties of SPR approach, localized surface plasmon resonance (LSPR) were introduced. LSPR, as an optical phenomenon, is produced by a light wave trapped within NPs. LSPR happens once surface electrons and incident light interact with each other. For LSPR, AuNPs and AgNPs are two common nanomaterials. Because of energy levels of d-d transitions, LSPR observes on spectra in visible range [44]. Employing LSPR phenomenon and Au nanorods, Park (2014) designed a label-free aptasensor with regeneration capability for the detection of OTA. This system is based on red shifts of LSPR wavelengths that are increased by binding of desire analyte to a OTA aptamer-coated gold nanorod substrate [45].

3.1.4. Lateral Flow-Based Aptasensors

Lateral flow devices, also called strip tests or immunochromatographic strips, rely on immunoreagent movement of an analyte-containing solution through a porous material [46]. A porous capillary membrane, and a receptor probe with read-out zone are major essential components of a standard lateral flow immunodiagnostic device. Lateral flow diagnostic tests can detect a wide range of substances such as mycotoxin, antibodies, and antigens. In general, lateral flow test strips benefit from a one-step detection approach for diagnostics. In addition, these test systems can be multiplexed for simultaneous determination of several mycotoxins on a single strip.

Various nanomaterials have been explored for immunodiagnostic test strips for improvement of sensitivity and selectivity. Owing to distinguished features, AuNPs have been used extensively as receptors in lateral flow test strips [47]. Currently, Ag nucleation on Au is an established method for signal amplification in biosensing systems [48]. Very recently, a competitive lateral flow immunoassay was developed for OTA detection relied on Ag deposition on AuNPs. Here, besides to signal amplification, AgNPs nucleation causes to reduce the amount of antibodies and competitors [47].

For on-site detection, an aptamer based lateral flow system has been developed for analysis of OTA. The preparation principle of the aptamer-based lateral flow strip is based on a competitive reaction between the sample containing OTA and the immobilized DNA probe 1 (test zone) for binding to the migrating AuNPs-aptamer conjugate. The solution was re-dissolved with the AuNPs-aptamer conjugate by capillary forces, and then the mixture migrated to the test zone. The DNA probe 1 captured the AuNPs-aptamer conjugate, allowing color particles to be concentrated and form a visible line in the test zone, resulting in two lines in the control and test zones, respectively. A positive sample with greater content of OTA than the cutoff level would compete with the DNA probe 1 and resulted in no visual line in the test zone, with only one line in the control zone. No matter for positive or negative samples, there would be a visual line in the control zone to ensure the effectiveness of the prepared strip, and if not, it may be invalid. This assay exhibited LOD of 1 ng/mL with no important cross reactivity. Whole detection time lasted for 15 min without any use of special instruments [49].

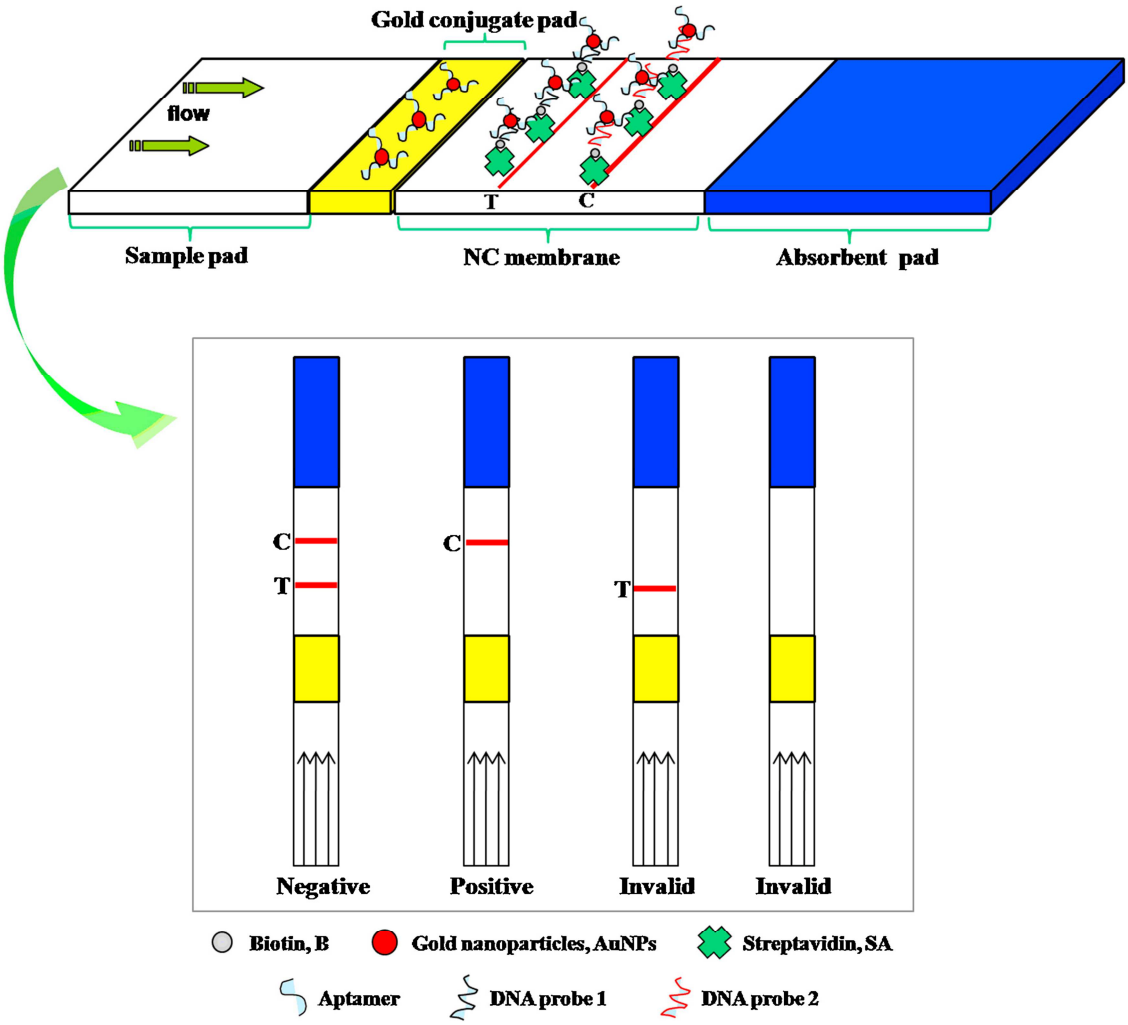

Figure 4. *Schematic illustration of aptamer-based lateral flow strip for detection of OTA [49].*

3.1.5. Fluorescence-Based Aptasensors

Fluorescence can occur when an excited dye/molecule/nanomaterial emits light upon returning to its ground state [36]. Owing to their extraordinary properties such as high sensitivity and ease of operation, fluorescence-based aptasensors are highly employed for detection purposes. Such aptasensors are mainly classified into labeled and label-free models [36]. Label-free aptasensors are the ones that do not require any chromophores or fluorophores for their detection performance, however, labeled aptasensors need at least one type of chemo/fluorophores. As labeled, there are different auto fluorescent molecules as well as synthetic ones. Additionally, advances in nanosciences have introduced some nanomaterials carrying fluorescence and quenching properties as alternative for dye labeled sensing systems.

However, labeling is a time-consuming and labor-intensive process and may change the binding affinity and selectivity of aptamers. Recently, label-free aptasensors including metal NPs, a basic-site-binding dyes, DNA intercalators, and aptamer-binding dyes, have attracted interests [50].

In the previous work of Taghdisi and his friends (2016), they successfully developed a sensitive and selective fluorescent labeled-aptasensor using AuNPs, silica NPs (SNPs), OTA aptamer as well as complementary strand of aptamer (CS) [51]. AuNPs and SNPs acted as fluorescence quencher and fluorescence intensity enhancer, respectively. In the absence of target, no carboxyfluorescein (FAM)-labeled biotinylated CS remained in the environment of SNPs-coated with streptavidin. Thus, no fluorescence emission was observed. In the presence of OTA, the aptamer on the surface of AuNPs captured the OTA and released the FAM-biotin-CS. Thus, it could be attached to streptavidin-SNPs followed by occurrence of remarkable fluorescence emission. With a LOD of 98 pM, the fabricated platform selectively detected OTA. In another step, we applied it to detect OTA in serum and grape juice with LODs of 0.152 and 0.113 nM, respectively [51]. The developed aptasensor provided a highly sensitive platform for OTA detection.

More recently, Nameghi and his group (2016), they designed another fluorescent aptasensor for sensitive and specific detection of OTA with LOD of 0.135 nM. The sensor was based on a DNA pyramid nanostructure (DPN) and PicoGreen (PG) dye. PG was used as fluorescent probe because of high sensitivity and specificity. Interestingly,

when OTA was present in the analyte-containing solution, the special nanostructure of DPN was disassembled. In this state, after addition of PG, weak fluorescence emission was detected as the double strand DNA binding site for PG was remarkable reduced due to attachment to OTA. In the absence of the analyze, the pyramid structure and the double strand DNA molecules remained intact. Thus, after addition of PG, intensive fluorescence emission was observed. This analytical technique can detect OTA in grape juice and serum with LODs of 0.149 and 0.184 nM, respectively [52]. The aptasensor had very interesting and novel design as well as high sensitivity.

In a similar approach, C. Wang et al. (2015) designed an aptasensor with LOD of 3 nM, using evanescent wave all-fiber (EWA). The applied strategy was based on aptamer-modified magnetic beads (MB-Ap), FAM-labeled streptavidin (SP)-conjugated CS oligonucleotides as signal probes (SP) and a dethiobiotin-modified fiber which is embedded inside the evanescent wave biosensor system. In the presence of OTA, the MB attached aptamer released the FAM-SP-CS and captured the free OTA. Hence, the released FAM-SP-CS could attach to the dethiobiotin-modified fiber and induced fluorescent signal [16]. As mentioned earlier, one of the most important challenges in systems which rely on immobilizing a probe on a solid surface is to keep the integration of nucleic acid surface after repeated measurements. Wang et al. (2015), addressed this challenge and apart from the versatile features such as portability, sensitivity, the present EWA-based aptasensors show high stability and significant regeneration capability over other aptasensing models. Their proposed strategy showed linear responses ranging from 6 nM to 500 nM [53].

In an interesting and different approach, Wei et al., introduced a fluorescent based aptasensor for OTA using graphene. Sensing methods employing single wall carbon nanotubes (SWCNTs) and graphene oxides have presented sensitive and selective performance; In addition, desired effects of nano-graphite, including high surface area and unique mechanical, electronic and thermal properties, have attracted great attention in using this material for sensing applications. The π-rich structure of nano-graphite makes it as a superior quencher, and exhibits low background and high signal-to-noise ratio. For the first time, a novel system employing nano-graphite-aptamer hybrid and DNase I, was introduced for OTA analysis. FAM-labeled OTA aptamer was attached on nano-graphite surface. Nano-graphite, as quencher, absorbed fluorescence emission raised from FAM. Briefly, in the presence of OTA, the FAM-aptamer released the graphene and attached to small OTA and fluorescent signal of FAM became observable. Additionally, DNase I could digest the aptamer, thereby liberating the fluorophore and ultimately releasing the OTA. The released OTA then binds to another aptamer on the nano-graphite, resulting in the successive release of distinguishable signal dye-labeled aptamers from the nano-graphite, which leads to significant amplification of the signal. LOD of this amplified sensing system was measured as 20 nM. Ease of production, cost-effectiveness, homogeneous detection and applicable for conducting high-throughput assays are some advantages offered by this platform [54].

Moreover, as another carbon-based material single-walled carbon nanohorn (SWCNH) were also used for development of OTA fluorescent aptasensors. SWCNH is a cost-effective, metal free and new form of carbon nanomaterial. It consists of single-wall graphene sheet which acts as a horn-shaped sheath. Due to its spherical structure, when in aggregate form, and horn shape form, SWCNHs have been extensively used in biosensors, adsorption and drug delivery applications. Using aggregation property of SWCNHs, a novel fluorescent sensing platform was designed for the detection of OTA present in red wine samples. SWCNHs played as quencher in this system. In the absence of OTA, after adsorption of FAM-modified aptamer onto SWCNH surface, the fluorescence resonance would be quenched owing to proximity of fluorophore and quencher. In the presence of OTA, G-quadruplex would form and fluorescence of FAM could not be quenched. In proportion to OTA concentration, fluorescent intensity would increase. Linear detection range and LOD of this strategy were 20–500 nM and 17.2 nM, respectively. Recoveries of spiked samples calculated from 93% to 104.9%. Quick and simple determination at room condition is of advantages of this procedure [55].

Interestingly, polymerase chain reaction (PCR) can also be applied for fluorescent based OTA aptasensor regarding its enabling property for signal amplification. Signal amplification is critical to conduct accurate measurements with reliable results. However, PCR suffers from some inherent issues including false positive results, labor-intensive assays and complicated thermal cycling steps, which restrict its extensive use for biosensing. Owing to simplicity and high efficiency, rolling circle amplification (RCA) has been employed for biosensing applications. In comparison with PCR, RCA has a lower sensitivity. To overcome this issue, hyperbranched rolling circle amplification (HRCA) has been developed as an alternative to RCA. By combining the latter with the specific function of aptameric recognition, a simple, selective and sensitive aptasensor was designed for OTA determination in food samples. OTA bound to aptamer which inhibited hybridization reaction between capture probe DNA (CDNA) and aptamer. Then, HRCA reaction would be initiated after hybridization of CDNA with padlock probe. Finally, HRCA could combine with SYBR green I which resulted in fluorescent signal production. There was a linear relationship between logarithm concentration and fluorescence intensity in the range of 4 fg/mL to 400 pg/mL. LOD in this system was determined as 1.2 fg/m [56].

Consequently, fluorescent dyes suffer from some inherent issues such as short lifetime and stability which inhibit their use for on-site analyses. Fluorescent NPs have been developed with improved signal strength and ease of functionalization as alternatives for fluorescent dyes. Furthermore, integration of fluorescent NPs with magnetic NPs have been developed to improve the analytical

performance of fluorescent-based methods. Magnetic NPs are highly used for separation and extraction purposes during sensing owing to their high surface area. Combining the above-mentioned RCA approach with fluorescent NPs and magnetic beads (MBs), a competitive method was achieved for ultrasensitive detection of OTA with LOD of 0.13 ppt. An effective magnetic separation system could enhance signal-to-noise ratio, while the RCA would provide many hybridization sequences for the QDs labeled probe, leading to increase in response signal. In this platform, the specific OTA aptamer is synthesized as a segment of RCA primer. Then, this aptamer is immobilized on the MNPs surface. In the presence of OTA, RCA process would be inhibited due to higher binding affinity of primer with OTA than RCA. But, in the absence of target, after hybridization of primer with padlock, it would be produced through repeated sequences. Increasingly, the prolonged ssDNA hybridized with several QDs labeled DNA probes. Finally, the variation of signal was significantly intensified. This model showed linear response for the range of 10−3 to 10 ppm of OTA. With some changes, this model can be applied to other fields. Application of QDs and magnetic purification eliminated previous difficulties for RCA characterization via gel electrophoresis and repeated washing step, respectively [57].

Taking advantage of fluorescent NPs, another OTA aptasensor was developed using caboxyl-modified fluorescent particles and magnetic particles. The sensor employed displacement and competitive format which provided IC50 and LOD of 7.2 nM and 0.005 nM for OTA, respectively [58]. In displacement assay, the fluorescent particles-modified aptamer was detached from the surface of OTA-modified MBs in the presence of free OTA in the binding medium. The varying concentrations of free OTA can be related to the detaching extent of the fluorescent particles-modified aptamer. In case of an indirect competitive assay, OTA-MBs were immobilized inside the wells and the analysis was performed by adding the fluorescent particles-modified aptamer which competed with the immobilized OTA and OTA in solution. The presence of OTA in solution prevented binding of the immobilized OTA to the aptamer, leading to a decrease of the fluorescence signal.

However, elimination of receptor molecules from the solid immobilization surface and also change of single-stranded DNA conformation are inevitable for sensors which rely on immobilization of nucleic acid molecules. Thus, such sensors are not suitable for multi-use applications, and the receptor molecules should be regenerated over the immobilization surface. Although, some chemical and thermal methods have been suggested for regeneration of DNA biosensor, there are only a couple of effective methods to address these issues. Furthermore, there are some other nanomaterials-based fluorophores beside QDs. DNA-scaffolded silver-nanocluster (AgNCs), as novel fluorophores, have shown high performance for DNA-based biosensing. They benefit from an appealing set of properties including ease of synthesis, biocompatibility, high photostability and outstanding quantum yield [59]. Based on above studies, a signal-on fluorescence aptasensor, was developed for ultrasensitive detection of OTA in wheat samples with high specificity. LOD of this procedure was as low as 2 pg/mL. In this developed biosensing assay, the DNA-AgNCs photoluminescence emission band can be changed by replacement the DNA sequence. Thus, this method can be developed for the detection of other mycotoxins [59].

3.2. Electrochemical-Based Aptasensors

Electrochemical sensors are based on the detection of changes in electric current produced by redox reactions occurring on electrode surface. In general, application of aptamers as bio-recognition elements is of great interest in electrochemical detection methods. Electrochemical aptasensors combine aptamer with electrochemical transducers to produce an electrical signal for analysis of an analyte. It is mainly based on measurement of electrical potential or current changes produced by interactions happening over the transducer surface. Electrochemical biosensors benefit from ease of operation, small size, high selectivity and sensitivity. Contrary to optical instruments, electrochemical detection methods are cost effective and more feasible for on-site application since they require fewer reagents or ancillaries [58].

Recently, different electrochemical aptasensors were developed for the detection of OTA [60], [61]. A selective and sensitive impedimetric aptasensor was exploited for detection of OTA in cocoa beans. This is the first report that introduced a label-free and facile electrochemical model.

In that study, anti-OTA aptamer was immobilized on screen printed carbon electrodes (SPCEs), based on diazonium-coupling reaction for detection of OTA in cocoa beans. LOD was determined as low as 0.15 ng/mL. Recovery percentage varied from 91% to 95% with RSD of 4.8% in cocoa samples. Within the range of 0.15–2.5 ng/mL, a linear correlation was found between OTA concentration and electron transfer resistance. Benefiting from high stability and reproducibility, this platform can be used for monitoring of cocoa in chocolate industries and collection centers [62].

A novel and label-free electrochemical sensing strategy was proposed for OTA detection in rice samples [63]. In this system, two-piece macromolecules (NH_2-PEG-Anti-OTA-aptamer) were grafted on a boron-doped diamond microcell, forming a dense layer. In the absence of analyte, electron transfer to electrode surface was performed, owing to unfolded conformation of aptamer. However, addition of the analyte resulted in changing of aptamer structure to the G-quadruplex. In the latter conformation, electron flow was blocked. The increasing OTA concentration results in electrochemical signal reduction. For the OTA range of 0.01–13.2 ng/L, a linear plot was obtained. In addition, LOD was determined as 0.01 ng/L. In addition, the sensor showed high sensitivity with reduced complexity operation in comparison with similar sensors [63]. Importantly, the aptasensor could overcome the challenge of label-free detection of small size molecules through aptamer formation.

Furthermore, based on differential pulse voltammetry, a

competitive aptasensor was developed for OTA screening in cocoa samples. The specified molecular imprinted polymer columns were employed for extraction and purification of OTA. On the other hands, the amino modified aptamers were immobilized onto the SPCE. Using competitive behavior exists between target analyte and biotin labeled for the binding site on the aptamer, the binding percentage was determined. LOD and relative standard deviation (RSD) were obtained 0.07 ng/mL and 3.7%, respectively. The proposed model exhibited a good linearity ranging from 0.15 to 5 ng/mL. Also, recovery percentage was determined in the range of 82.1–85%. Advantages such as fast response, easy operation, high sensitivity and selectivity make this system feasible for one-site screening applications [64].

Two-dimensional nanomaterials have been recognized as promising components for sensing, because of remarkable electrochemical conductivity, high surface area and acceptable chemical stability. These substances have high signal amplification activity. For example, $MoSe_2$ has showed good electrical conductivity and was found to be beneficial for sensing applications. Owing to response enhancing and high conductive activity features, AuNPs are also recommended for detection applications. Taking advantage of $MoSe_2$ properties, Huang et al. (2016) developed an electrochemical biosensor for sensitive detection of OTA in wine samples with LOD of 0.08 pM. $MoSe_2$ nanoflowers were synthesized employing hydrothermal technique. cDNA and methylene blue were used for signal amplification and as tracer, respectively. The proposed platform integrated $MoSe_2$ and AuNPs onto electrode surface. Then, the OTA aptamer was coated onto the modified electrode. In the absence of OTA, CS oligonucleotide could be hybridized with the aptamer and more MB could be attached to the DNA sequence and produce electrochemical signal. However, in the presence of OTA, CS oligo was released and reduction in MB signal could be observable. Using Au-S interaction, OTA aptamers were immobilized onto the modified electrode surface. AuNPs and $MoSe_2$ were used for modification of the electrode. Remarkably, this assay presented a good linear correlation ranging from 0.0001 to 1 nM. Recovery and RSD values were obtained in the range of 96.2–104.3% and 2.8–4.5%, respectively [65].

Via integration of DNase I-based target recycling reaction with aptamer-graphene nanosheets, a simple and feasible aptasensing protocol was developed for monitoring of OTA in contaminated wheat samples. Owing to strong binding of graphene oxide nanosheets with nucleic acid, aptamers were attached to its surface. In the absence of target, thionine-aptamers were attached on the graphene oxide nanosheets, while OTA addition resulted in dissociation of them. In the next step, DNase I readily reacted to thionine-aptamer/OTA structure and cleaved it. Released OTA molecules, retriggered the above-mentioned reaction that led to the production of numerous free thionine molecules. Electrical signals were derived from screen-printed carbon electrode (SPCE) (negative charge) by thionine molecules. Proportionally, increasing of target concentration enhances current signal. Under optical condition, this system is able to detect OTA concentrations of as low as 5.6 pg/mL. In comparison with other methods, present platform does not need to have multiple washing steps, sample preparation as well as immobilization aptamer on the sensing interface [66].

In another work, graphene-oxide nanoplatelets (GONPs), as inherently electroactive labels, were used in the design of an electrochemical aptasensor for OTA detection. The fabricated system was able to detect OTA molecules in the range of 310 fM to 310 pM. To fabricate the sensor, OTA aptamers were immobilized onto the electrode surface. It was showed that after incubation with GONPs, these substances were conjugated with immobilized aptamers through π-π interactions. On the other hand, when OTA is present in the solution, it specifically can be attached to the aptamers. This makes some conformational changes which results in the removal of some immobilized aptamers from the electrode surface. Subsequently, when modified electrode was incubated with GONPs, these nanoplatelets adsorbed onto the remained immobilized OTA aptamers. Thus, signal level would be reduced in the presence of OTA [67].

By using Iridium oxide nanoparticles (IrO_2 NPs), a novel and label-free platform was introduced for OTA detection. In this model, electrode surface was modified with polythionine (PTH). Then, IrO_2 NPs were adsorbed onto the electrode. After this, selective OTA aptamers were immobilized through electrostatic interaction. Addition of OTA to this system resulted in aptamer conformation change followed by electrochemical change. LOD in this impedimetric aptasensor was reported as 14 pM. Linear correlation observed in the range of 0.01–100 nM. In addition, recovery percentage was determined between 118 to 133% [68].

4. Conclusion

The present review summarizes the new developed fluorescence detection device and new published sensors which employed aptamer-based biosensors for sensitive detection of ochratoxin A (OTA). Due to noticeable toxicity of OTA, a sensitive and selective detection of this mycotoxin is necessary. In comparison with other developed methods, fluorescence analysis and aptasensors exhibit special features like high sensitivity, high selectivity, and are simple. In our days both fluorescence analysis and optical or electrochemical aptasensing models are used for OTA detection in food samples. Electrochemical biosensors are easy to operate with small size, high selectivity and sensitivity. Contrary to optical instruments, electrochemical detection methods are cheap and more feasible for on-site application as they require fewer reagents or ancillaries.

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