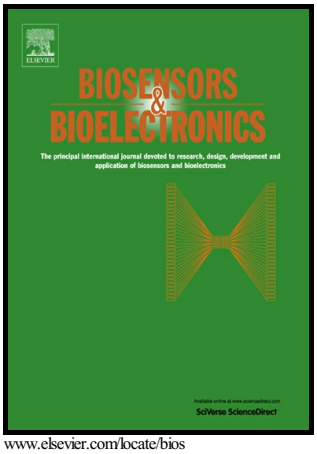

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Recent Advances in Mycotoxins Detection

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Abstract

Mycotoxins contamination in both food and feed is inevitable. Mycotoxin toxicity in foodstuff can occur at very low concentrations necessitating early availability of sensitive and reliable methods for their detection. The present research thrust is towards the development of a user friendly biosensor for mycotoxin detection at both academic and industrial levels in replace of conventional expensive chromatographic and ELISA techniques. This review critically analyze the recent research trend towards the construction of immunosensor, aptasensor, enzymatic sensors and others for mycotoxin detection with a reference to label and label free methods, synthesis of new material including nano dimension, and transuding techniques. Technological aspects in the development of biosensors for mycotoxin detection, current challenges and future prospects are also included to provide a overview and suggestions for future research directions.

Keywords: Immunosensor; Mycotoxins; Aptamers, Molecularly Imprinted Polymers Mimotopes, Enzymatic inhibition.

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Contents

1. Introduction

1.1 Origin of concept

The word mycotoxin originated from the Greek word mukos meaning "fungus" and latin word toxicum referring "poison" is secondary metabolite produced by fungus, that colonizes on crops either during harvesting or during storage **(Robbins et.al. 2000)**. The consumption of contaminated food causes severe toxic effects on humans and animals health **(CAST 2003, Zheng et.al 2006, Rodrigues et.al. 2011)** and they are more dangerous than food additives or pesticide residues (**Van Egmond et.al. 2007.** They are teratogenic, mutagenic, nephrotoxic, immunosuppressive and carcinogenic. They are highly resistive in nature and hence remain in the food chain. International Agency for Research on Cancer (IARC) has categorized aflatoxin B1 (AFB1) as Group 1 (potent human carcinogen) and may be responsible for human liver cancer in many developing countries **(Henry et.al. 1999)** while ochratoxin A (OTA) and fumonisin are classified as Group 2B carcinogens (possibly carcinogenic in humans) **(IARC 2002)**. Therefore, European commission has set maximum permitted levels for certain mycotoxins in a majority of foods (**Table S1**). Low molecular weight mycotoxins posses variety of chemical structures (**Fig. S1**) which is the main constraint to develop one standard technique for analysis **(Tamerler et.al. 2006)**.

There has been a major international research effort, aimed at the identification and quantification of mycotoxins and evaluation of their biological effects in humans and animals (**Zain et.al. 2011**). The main group of moulds and mycotoxins of world-wide concern are *Aspergillus* spp. (producing aflatoxins B1, B2, G1, G2, OTA and patulin), *Fusarium* spp. (producing T-2 toxin, deoxynivalenol (DON), zearalenone and fumonisin B1) and *Penicillium* spp. (producing OTA).

1.2. Conventional techniques used for the detection of mycotoxins

Conventional analytical methods for mycotoxin detection include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV), diode array (DAD), fluorescence (FD) or mass spectrometry (MS) detectors, gas chromatography (GC) coupled with electron capture (ECD), flame ionization (FID) or MS detectors (**Lippolis et.al. 2008, Visconti et.al 2005**) and enzyme-linked immunoassay (ELISA) (**Wang 2011, Zheng et.al. 2005)**. Though these methods are well known for their accurate and precise detection of mycotoxin in food or feed samples, they require skilled operators, extensive sample pretreatment, equipment and may lack accuracy at low analyte concentration **(Cigić & Prosen 2009, Goryacheva et.al. 2007).** Therefore, a rapid, sensitive and specific assay technique is required for the routine analysis of foods, and beverages. The main objective is to review the research work carried out in process of development of an efficient user friendly and reliable biosensing tool which can quantify the amount of mycotoxins in our daily diet.

2. Recent trends towards the detection of mycotoxins

The recent advanced techniques such as biosensors have focused on some general features such as simplicity, easy-to-use, relatively fast and easy portability. The receptors developed for mycotoxin detection are biomolecules (antibodies, DNA and enzymes) and synthetic chemicals (aptamers, MIP, mimotopes etc). Being small and neutral molecules, mycotoxins can be better detected through labels like enzymes, nanoparticles, redox molecules and fluorophores etc. Therefore, the developed sensors are categorized in two groups like labeled **(Fig 1a)** and label-free sensors **(Fig.1b)** which can be further divided into competitive and non-competitive on the basis of detection strategy. In the present review, emphasis has been given on

the material construction of the transducer matrix along with the detection principle. Based on the type of receptor, biosensors for mycotoxin detection are described as below:

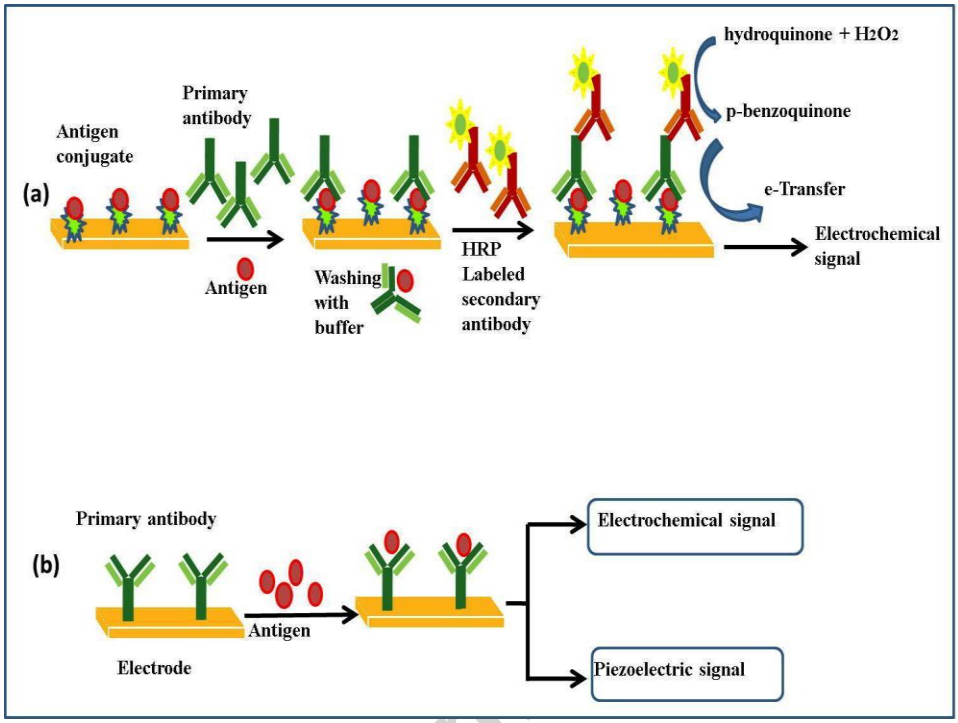

Fig.1a: Labeled detection of antigen**. (b):** Label free detection of antigen.

2.1. Immunosensors for mycotoxin

2.1.1. Labeled Immunosensor

The detection of small analytes (low molecular weight molecule) is mostly conducted using a competitive immunoassay format. In this assay, the sample analyte competes with coated labeled analyte or analyte-protein conjugate for a limited number of antibody binding sites. As the analyte concentration in sample increases, more labeled analytes are displaced **(Prieto-Simón et.al. 2012, Pemberton et.al. 2006, Piermarini et.al 2007**). In the competitive assays, two types of protocols are followed. In the first mode, the surface of electrode is coated with the saturated concentration of labeled analyte-protein conjugate and competition takes place between

free anylate and immobilized labeled analyte-protein conjugate for the fixed amount of antibody named as "indirect" method. The most common analyte-protein is found to be BSA-mycotoxin conjugate. Sometimes, a linker molecule such as polyethylene glycol (PEG) is employed as a spacer in between OTA and ovalbumin (OVA) conjugate (OTA-PEG-OVA) to reduce the steric effect (**Yuan et.al. 2009).**

Recent research work demonstrates that the nanomaterial or quantum dots (QDs) modified immobilizing matrix, coated antigen or secondary antibody can enhance the response signal (**Bonel et.al. 2010, Liu et.al. 2009**). A signal amplification of surface plasmon resonance (SPR) by a factor of more than 10 has been achieved using AuNPs labeled secondary antibody in a sandwich mode as compared to AuNPs tagged with primary anti-OTA and results in the 60 pg/mL as limit of detection of OTA **(Urusov et.al. 2011).** Gan et al. have employed magnetic Fe_3O_4-graphene oxides (Fe-GO) as the absorbent and antibody-labeled with cadmium telluride QDs (CdTe QDs) as the signal tag for aflatoxin M1(AFM1) detection and achieved the LOD of 0.3 pg/mL (S/N = 3) (**Gan et.al. 2013**). With the introduction of upconversion nanoparticles (UCNPs), Wu et.al. **(Wu et.al. 2011)** have introduced an immunoassay for simultaneous fluorescence detection of AFB1 and OTA in food samples.

In the direct mode, free sample analyte competes with the absorbed labeled analyte-protein conjugate for the fixed amount of immobilized antibody. The transducer monitors the label concentration and hence measure the target analyte concentration, present in the solution. Alarcon et al. (**Alarcon et.al. 2006)** have observed that direct method is more sensitive than indirect method for OTA estimation.

The sensitivity of quartz crystal microbalance (QCM) transducer can be enhanced for sensitive analysis of low molecular weight mycotoxin using sandwich format through labeled secondary antibody. Jin et.al. have enhanced the sensitivity using the oxidative label HRP, that oxidized 4- chloro-1-naphthol into insoluble product (benzo-4-chlorohexadienone) and AuNP tagged secondary antibody as mass inducer over the crystal **(Jin et.al. 2009a, 2009b)** and achieved a linear range of 0.01-10.0 $ngmL^{-1}$ for the determination of AFB1 in buffer or sample solutions**.**

The use of magnetic bead (MBs) micro/nanoparticles in the separation process offers great advantages such as easy handling, reusability, homogeneous dispersion and a great surface area**(Aguilar-Arteaga et.al. 2010, Vidal 2012)**. The magnetic particles have helped in separations with efficiency close to 100 % with complete removal from interfering substances (**Prieto-Simon et.al. 2008**). Identical approach is followed by Parrota et al. for the estimation of OTA from untreated red wine samples with the LOD of 0.008 ppb level without magnetic separation **(Parrota et.al. 2012**). Multiplexed, competitive immunoassay-based detection of different mycotoxins and the feasibility of running sandwich and competitive immunoassays formats on a single waveguide surface (**Sapsford et.al. 2006**) have been reported.

Labeled non-competitive immunoassay often follows the sandwich format and the enzymes/fluorescent material/nanoparticles tagged secondary antibody generates signal on capturing the antigen. Masoomi et al. **(Masoomi et.al. 2013)** have designed a sandwiched type electrochemical immunosensor for AFB1 estimation. The oxidation of catechol to o-quinone attached to secondary antibody via gold-coated iron nanoparticles is trapped for signal generation and iron nanoparticles offers regeneration of immunosensor using an external magnetic field. Though, the above immuno-platform has achieved a wide linear range of 0.6–110 $ngmL^{-1}$ with a

LOD of 0.2 ngmL^{-1}, the construction of sensor architecture is complex in nature. A zeranol immunosensor comprising of multiple labels such as sodium montmorillonites clay NPs (Na-Mont), thionine (TH), HRP attached to the secondary anti-zeranol antibody (Na-TH-HRP-Ab2) and Na-Mont NPs modified nanoporous gold (NPG) as an electrode material has been fabricated to detect Zearalenone up to 3 pg/ml (**Feng et.al 2013**).

2.1.2. Label-free immunosensor

The innovation of label free immunosensors can also be classified as non-competitive and competitive. The main advantage of these label free immunosensors is simplicity and single-stage reagent less operation. However, such immunosensors are often inadequate to meet the demand of sensitive detection. As concern of simplicity and speed of immunosensors, the non-competitive protocol is favored over the competitive one. The major drawback of noncompetitive label free immunosensor is false positive response due to nonspecific binding.

In a label free non-competitive immunosensor, the interaction of immobilized antibodies and analyte is read out by the transducer. The transducer and mode of detection play the important role for obtaining the enhanced sensitivity of the label free immunosensor. The sensing ability of an electrochemical immunosensor can be enhanced by incorporating either a metal (PtCo NP) or metal oxide nanoparticles(such as CeO_2, Fe_3O_4, TiO_2, ZnO etc.) or conducting polymer etc into a given matrix (**Sharma et.al. 2010, Srivastava et.al. 2013, Solanki et.al. 2009, Feng 2013**).

Compared to the amperometric immunosensors, electrochemical impedance spectroscopy (EIS) based immunosensors have been found to be more sensitive and reliable. EIS studies investigate the electrical properties of the interface of sensing device and trace the reactions

occurring on its surface (**Radi et.al. 2009, Zamfir et.al. 2011**). The EIS technique coupled with online flow detection tool have been found to lower the detection limit of AFM1 and AFM2 up to a level of 1 pg/mL in drinking yogurt and flavored milk (**Kanungo et.al 2014**) as compared to lateral flow system (**Andreou and Nikolelis 1998**). EIS immunosensing platform based on 11-mercaptoundacnoic acid (11-MUA) self-assembled on silver wire has detected AFB1 in groundnut extract with the LOD of 0.01 $pgmL^{-1}$ and AFM1 detection (6.25-100 $pgmL^{-1}$) in milk (**Bacher et al. 2012**).

Using the fluorescence resonance energy transfer (FRET) mechanism Li et al. have demonstrated a label-free non-competitive immunoassay while antibodies with tryptophan (Trp) residues exited at 280 nm acts as a donor on interaction with antigen (AFB1). The fragment of antibody (Fab) promotes the detection of AFB1 about 10 times higher than the complete aAFB1 antibody (**Li et.al. 2013**). The label-free optical immunosensors based on the concept of reflection of light are found to be a promising approach **(Nabok et.al. 2007, Nabok et.al. 2005).** The gold bounded protein G (GBP-ProG) offers higher SPR signal intensity (**Park et.al. 2014)** in comparison to the ProG or thiol-modified surface (**Tamerler et.al. 2006**). Adyani et.al. have reported superiority of competitive mode (0.5-10 ng mL^{-1}) over non-competitive [5–10 $ngmL^{-1}$] using optical waveguide light mode spectroscopy (OWLS) technique for detection of AFB1 and OTA (**Adanyi et.al. 2007**). The application of nano material at an appropriate platform can overcome the challenge of label-free estimation of AFB1 (**Ah et.al 2012**). An optical approach with gold nanorods (GNRs) has been employed for the label-free competitive detection of AFB1 with the LOD of 0.16 $ngmL^{-1}$(**Xu et.al. 2013**). The disadvantages of these (optical) techniques include costly equipment, challenges of regenerating the sensor chip and non specific adsorption.

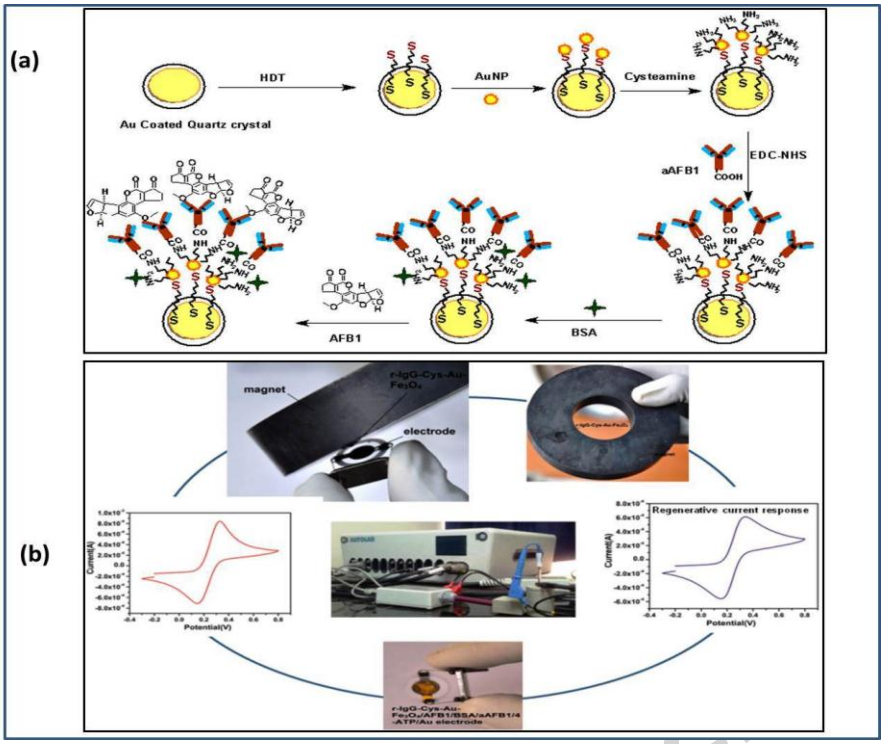

Fig.2 (a): AFB1 detection using label free noncompetitive technique. **(b):** Schematic diagram represents the regeneration of sandwich type immunoelectrode for the detection of AFB1.

Label-free approach with QCM transducer is currently not popular due to small size of mycotoxins **(Cheng 2012, Tsai & Hsieh 2007, Vidal et.al 2009)**. QCM or Cantilever based technique is exploited for detection of AFB1 and OTA **(Ricciardi et.al. 2013).** Chauhan et.al. (**Chauhan et.al. 2015a, Chauhan et.al. 2016**) have developed label free electrochemical quartz crystal microbalance (EQCM) immunosensor based on AuNP coated self assembled monolayer with a LOD of 8 $pgmL^{-1}$ for AFB1 detection **(Fig. 2a**). While gold coated Fe_3O_4 nanostructure

tagged secondary polyclonal antibodies have been exploited in a competitive sandwiched format

SN	Matrix	Electrode	Type of Ab	Mode of detection	Label	Technique	Toxin	Response range	LOD	Sensitivity	Relative Standard Deviation (RSD)in %	Coefficient of variation (CV)in %	Reference

to develop a reusable immunosensor using strong external magnet (**Fig.2b**) (**Chauhan et al.2015b**). A brief summary of developed immunosensors for mycotoxin analysis are presented in Table 1.

Table 1: The characteristics of the reported labeled and label free immunosensors.

1	Graphite/ silver	**SPCE**	**mAb**	**Competitive**	**OTA-ALP**	**DPV**	**OTA**	**0.05-2.5ng/ml** **0.1-7.5 ng/ml**	**60 ng/ml** **0.4 ng/ml** **100 ng/ml**	**-**	**8-16**	**7**	**Alarcon et.al. 2006**
2	Graphite based ink	**SPCE**	**pAb**	**Competitive**	**Au-labeled ALP-Sec Ab**	**DPV**	**OTA**	**0.9-9 ng/mL** **0.3-8.5 ng/mL**	**0.86 ng/mL** **0.20 ng/mL**	**-**	**8, 10**	**6**	**Bonel et.al. 2010**
3	**Nitrocellulose Membrane**	**-**	**pAb**	**Competitive**	**Au coloids -pAb**	**Lateral Flow immunoassay**	**OTA**	**1-10 µg/kg**	**1.5 µg/kg**	**-**	**14-18**	**>20**	**Laura et.al. 2011**
4	**1,6-Hexanedithiol**	**Au**	**mAb**	**Competitive**	**ALP**	**Electrochemical (DPV)**	**OTA**	**10 pg/mL – 100ng/mL**	**8.2 pg/mL**	**-**	**-**	**-**	**Liu et.al. 2009**
5	**Magnetic beads with Protein G**	**SPCE**	**mAb**	**Competitive**	**HRP**	**SWV**	**OTA**	**0.01-20 ppb**	**0.008 ppb**	**-**	**5.56**	**-**	**Parrotta et.al. 2012**
6	**-**	**SPE**	**mAb**	**Competitive**	**OTA**	**-**	**OTA**	**0.3 ng/L-µg/L**	**-**	**-**	**20, 36**	**-**	**Prieto-Simon et.al. 2012**
7	**Poly(methyl methacrylate) (PMMA) beads**		**mAb**	**Competitive**	**HRP**	**-**	**OTA**	**-**	**0.7 ng/mL**	**-**	**-**	**-**	**Simon et.al. 2008**
8	**16-MHDA**	**Au**	**pAb**	**Competitive**	**Au NPs**	**QCM**	**OTA**	**10-128 ng/mL**	**8 ng/mL**	**-**	**-**	**8.96**	**Vidal et.al. 2009**
9	**Magnetic Beads**	**SPCE**	**mAb**	**Competitive**	**HRP**	**DPV**	**OTA**	**0.1 pg/mL-1µg/mL**	**10 pg/L**	**-**	**9**	**-**	**Vidal et.al. 2012**
10	**11-MUOH**	**Au**	**mAb**	**Competitive**	**Au NPs**	**SPR**	**OTA**	**-**	**1.5 ng/mL** **Au-0.042 ng/mL**	**-**	**-**	**-**	**Yuan et.al. 2009**
11	**-**	**SPCE**	**mAb**	**Competitive**	**Au NPs**	**Impedimetric**	**AFM 1**	**15 - 1000 ng/mL**	**15 ng/mL**	**-**	**20**	**-**	**Vig, et.al.**

													2009
12	MPA-BSA		mAb	Competitive	HRP	QCM	AFB 1	0.01–10.0 ng mL^{-1}	0.01 ng mL^{-1}.	-	4-7	-	Jin et.al. 2009
13			mAb		Fe_2O_3 nano particles	GMR	AFB 1	50pg/mL-50ng/mL	50 pg/mL	-	-	-	Mak et.al. 2010
							AFB 1 ZER HT-2	330pg/mL and 33ng/mL					
14		SPCE	mAb	Competitive	ALP	Electrochemical	AFB 1	0.1–10 $ngmL^{-1}$,	90 $pgmL^{-1}$.	-	18-22	-	Mmida et.al. 2006
15	GCE/PTH/Au NP AFB1-BSA-polythionine (PTH)/gold nanoparticles	GCE	pAb	Competitive	HRP	Electrochemical	AFB 1	0.6 - 2.4 ng/mL	0.07 ng/mL	-	2-5	-	Owino et.al. 2008
16			pAb	Competitive	ALP	Intermittent pulse amperometry (IPA)	AFB 1	0.05 and 2 ng/mL	-	-	7-10	-	Piermarini et.al. 2007
17	-	agarose-modified glass slides	mAb		Cy3		AFB 1 AFM 1 Deo	AFB 1 : 0.04–1.69 AFM 1: 0.45–3.90 Deoxynival	-	-	-	15	Wang et.al. 2012

							xynivalenol OTA T-2, ZON	enol: 20.20–69.23 OTA: 35.68–363.18 T-2 toxin : 0.11–1.81 Zearalenone : 0.08–7.47 ng/mL					
18	magnetic nanoparticles (MNPs)	-	mAb	Competitive	$NaY_{0.78}F_4$:$Yb_{0.2}$, $Tm_{0.02}$ and $NaY_{0.28}F_4$:$Yb_{0.7}$,$Er_{0.02}$ UCNPs	Flourescent	AFB1 OTA	0.01 to 10 ng mL^{-1}	0.01ng mL^{-1}	-	5.19 2.14	-	Wu et.al. 2011
19	Au/ 3-Mercaptopropionic acid (MPA)		mAb	Competitive	Au NPs	Piezoelectric	AFB1	0.1–100ngmL^{-1}	0.01 ng mL^{-1}	-	4-7	-	Jin et.al. 2009
20	MWCNT-GCE	GCE	mAb	Competitive	HRP	flow injection (FI) system	ZON	-	-	-	-	-	Pei et.al. 2013
21	heterobifunctionalsilane	-	mAb	Competitive	Fluorescein isothiocyanate label	fiber-optic immunosensor	FB1	10- 1000 ng /mL	10 ng /mL	-	3.9	-	Urraca et.al. 2005
22	-	-	mAb	Competitive	HRP	Flow-Through Immunosens	ZON	0.019 to 0.422 ng mL^{-1}	0.007 ng mL^{-1}	-	-	-	Wang et.al. 2010

						or							
23	Fe_3O_4-graphene oxides	SPCE	mAb	Non-competitive	CdTe-CNT QDs Conjugate	ECL	AFM 1	1.0 to 1.0 × 5 10 pg/mL	0.3 pg/mL	-	3.5	-	Gan et.al. 2013
24	AET- Au NPs	Au interdigitated electrodes (150nm thick)	mAb	Non-competitive	HRP	conductometric	AFB1	0.5 to 10 ng/mL	0.1 ng/mL at 3δ.		7	7.1	Liu et.al. 2006
25	GCE/chitosan/ Au nanoparticle (AuNP)/anti-Aflatoxin B_1 (a AFB_1),	GCE	pAb	Non-competitive	catechol–Au–Fe_3O_4 NPs	Electrochemical	AFB1	0.6–110 ng mL^{-1}	0.2 $ngmL^{-1}$	-	-	6.2, 5.7	Masoomi et.al. 2013
26	nanoporous gold films (NPG)	GCE	mAb	Non-competitive(NC)	Na-Mont-TH-HRP-Ab_2	Electrochemical	ZON	0.01–12 ng mL^{-1}	3 pg mL^{-1}	-	2.46-5.17	4.2-5.2	Feng et.al. 2013
27	Chitosan-iron oxide	ITO	pAb	NC	-	DPV	OTA		0.5 $ngdL^{-1}$	36µA $ng^{-1}dL^{-1}$	-	-	Kaushik et.al. 2009
28	Nano SiO2 – CH	ITO	pAb	NC	-	DPV	OTA	0.5-6 $ngdL^{-1}$	0.3 $ngdL^{-1}$	-	-	-	Kaushik et.al. 2009
29	Chitosan-PANI	ITO	pAb	NC		EIS	OTA	Upto 10 $ngmL^{-1}$	1.0 $ngmL^{-1}$	-	-	-	Raju Khan et.al. 2009
30	CS/TiO2(Chitosan)	-	pAb	NC	-	EIS	OTA	1-10 $ngmL^{-1}$		7.5 nM	-	-	Khan et.al. 2008
31	4-carboxyphenyl	Au	-	NC	-	EIS	OT	1- 20	0.5	-	3.7-5.5	-	Radi et.al.

	SAM						A	ngmL^{-1}	ngmL^{-1}				2009
32	BSA/AO-IgG/AUT/Au (SAM of AUT)	Au	pAb	NC	-	EIS	OTA	0.5-6 ng/dL	0.08 ngdL^{-1}	36.86 ohm ng^{-1}dL^{-1}	-	-	Solanki et.al. 2010
33	16MHDA	Au	pAb	NC	-	QCM	OTA	50-1000 ngmL^{-1}	16.1 ngmL^{-1}		-	64	Tsai et.al. 2007
34	Au/TA/Glu/BSA/MNP-Ab	Au	mAb	NC	-	EIS, CV	OTA	0.01-5 ngmL^{-1}	0.01 ngmL^{-1}	-	4.7	-	Zamfir et.al. 2011
35	BSA/aAFB1-C-AuNP/MBA/Au Gold	Au	mAb	NC	-	Voltametric	AFM1	10 – 100 ngdL^{-1}	17.90 ngdL^{-1}	0.45 µAng – 1 dL	-	-	Aditya et.al. 2010
36	anti- AFB$_1$ /MWCNTs/ITO	ITO	mAb	Non-competitive	-	Voltametric	AFB1	0.25–1.375 ngmL^{-1}	0.08 ngmL^{-1}	(95.2 µAng−1 mLcm^{-2})	-	-	Singh et.al. 2013
37	SAM of 11-MUA	Silver Wire	mAb	Non-competitive	-	Impedimetric	AFM1	(6.25–100pgmL^{-1})	1 pgmL^{-1}	6.25 pgmL−1	-	-	Bacher et.al. 2012
38	BSA/AbAFB1/n-Sm2O3/ITO	ITO	mAb	Non-competitive	-	Voltametric	AFB1	10–700 pg mL^{-1}	57.82 pg mL^{-1} cm^{-2},	48.39 µApg^{-1} mL cm^{-2}	0.39	-	Singh et.al. 2013
39	-	-	Fragment Ab	Non-competitive	-	FRET based Immunoassay	AFB1	-	0.85 and 0.09 ng mL^{-1}	-	-	-	Li et.al. 2013
40	Nano-sized gold hollow balls (NGB) with dendritic	Au		NC	-	QCM	AFB1		0.05 ng mL^{-1}		2.3, 5.8	3.8-5.1	Liao et.al. 2007

	surface												
41	Silica gel-ionic liquid-antibody	GCE	pAb	NC	-	EIS	AFB 1	0.1–10 ng mL^{-1}	0.01 ng mL^{-1}		1.7	-	Zaijun et.al. 2010
42	APTES , Silane, Protein G	-	mAb	NC	-	Microcantilever	Aflatoxins OTA 1	Aflatoxins ; 3 $ngmL^{-1}$ OTA 1 : 6 ng mL^{-1}	-	-	-	-	Ricciardi et.al. 2013
43	RGO/ITO	ITO	mAb	NC	-	Electrochemical	AFB 1	-	0.12 ng mL^{-1}	68 µAng $^{-1}$ mL^{-2} cm	-	-	Srivastava et.al. 2013
44	DSP (3,3'-Dithiodipropionic-acid-di-N-hydroxysuccinimide ester)	Au	mAb	NC	-	QCM	AFB 1	0.5 - 10 ppb	-	-	-	-	Spinella et.al. 2013
45	Fe_3O_4/SiO_2 composite nanoparticles	Au	mAb	NC	-	QCM	AFB 1	0.3– 7.0 ng mL^{-1}	0.3 ng mL^{-1}	-	8.0	6.3	Wang et.al. 2009
46	Au/DDT SAM	Polycrystaline gold electrode	mAb	NC	-	TIRE and QCM	AFB 1	100 µgmL^{-1} to 0.15 ng mL^{-1}		-	-	-	Nabok et.al. 2007
47	CS–PtCo–Ab/Graphene-Thionine/GCE	GCE	mAb	NC	-	Electrochemical	ZON	0.05 to 5.0 ng mL^{-1}	13 pg mL^{-1}	-	4.5	-	Feng et.al. 2013
48	1-dodecanethiol (DDT)	Au	pAb	NC	-	Electrochemical	alter toxin I (ATX-I)	-	4 x 10^{-8} $moldm^{-3}$ (14 ppb)	-	7	-	Moressi et.al. 2007

49	-	-	mAb	Com/ NC	-	OWLS	AFB 1	5-10ngmL^{-1}	-	-	-	-	Adányi et.al. 2007
							OTA	0.5-10ngmL^{-1}					
50	Au NPs	-	-	Competitive	-	SPR	OTA	0.1 ngmL^{-1}-0.1 µgmL^{-1}	60 pgmL^{-1}	-	-	-	Urusov et.al. 2011
51			mAb	Competitive	-	EIS	AFM 1	1-100pgmL^{-1}	1pgmL-1		-	-	Kanungo et.al. 2014
52	Au NPs	Si-FET channel	mAb mAb mAb	Competitive	-	Si-FET	AFB 1	2.5 – 20 ngmL^{-1}	-	-	-	~11	ChilSeong et.al. 2012
							ZEN	0.5 – 50 ngmL^{-1}	-				
							OTA	0.5 - 100 ngmL^{-1}	-				
53	GNRs	-	mAb	Competitive	-	Optical	AFB 1	0.5 to 20 ng mL^{-1}	0.16ng mL^{-1},	-	-	-	Xu et.al. 2013

2.2. Aptasensor for the mycotoxin detection

The key part of the aptasensor is the aptamer, a synthetic oligonucleotide ligand (either single stranded DNA (ssDNA) or RNA containing 10–50 variable bases and are known to exhibit high specificity and strong binding affinity (**Cho et.al. 2009, Willner and Zayats 2007)**. Aptamers can fold into well-defined three-dimensional structures and bind to their ligands by complementary shape interactions (**Hermann and Patel 2000**). In comparison to the antibody,

an aptamer has several advantages such as ease of synthesis and modification with a variety of chemical groups, stability, cost-effectiveness etc (**Schmidt et.al. 2004, Yang et.al 2013).** The aptamers can maintain their structures over repeated cycles of denaturation and show high binding affinities and specificities towards the broad range of target (macro to micro molecules, like toxins, drugs, peptides, proteins and whole cells etc.). The aptamers are thus, considered to be a better alternative to antibodies in many biological applications. In 2008 Cruz-aguado and Penner have briefly studied the aptamer sequence (see supplementary file **Table S2**) for OTA (Cruz-aguado & Penner 2008) using *in-vitro* process SELEX.

2.2.1. Factors affecting aptamer affinity

2.2.1.1. Presence of divalent cation

The binding of OTA to the DNA aptamers depends on the presence of divalent cations. It is observed that negatively charged DNA forms a coordination complex with OTA (**Yang et.al. 2013, Yang et.al. 2011, Bonel et.al. 2011**) via carboxyl and 8-hydroxyl groups in presence of calcium or magnesium ions. It is shown that the aptamer has rather high affinity to OTA in the presence of 20 mM Ca^{2+} (dissociation constant Kd=49 nM) (**Cheng et.al. 2012**). Circular dichroism and spectroscopic experiments reveal that, on addition of OTA, aptamer changes from random coil structure to compact rigid antiparallel G-quadruplex structure (**Yang et.al. 2011**).

2.2.1.2. Effect of pH

The pH is known to affect the binding affinity. Aptamer shows high affinity at pH 7.0. In acidic pH, the 8-hydroxyl group in OTA is protonated which in turn inhibits the complex formation resulting in decreased binding to the aptamer. Aptasensors can be divided in two categories labeled and label-free as described below:

2.2.2. Labeled Aptasensors

The signaling in aptasensors occurs due to changes in the conformation of aptamer as the target (mycotoxin) molecules are introduced into the reaction chamber. The detection can be achieved through transducer either in "signal on" or "signal off" form, depending on the format of the assay **(Fig.3)**. Commonly used labels are fluorescence material (fluorescein, luminols, QDs etc), enzyme HRP, GO, ALP, electroactive compounds (ferrocene, ferro- cyanide, methylene blue (MB), Pt and Cds QDs, and other metal nanoparticles (NPs)). In the "signal on" format, target detection is based on the signal enhanced after the interaction with target. While the signal off refers to diminish in signal due to the formation of target-aptamer complex.

2.2.2.1. Signal On

"Signal On" format is represented by the scheme (**Fig. 3a).** The fluorescent labeled aptamer, and quencher moiety (QDNA) has been used to detect OTA with a linear dynamic range of 1-100 $ngmL^{-1}$ (**Cheng et.al. 2012**). Carbon nanomaterials like graphene, single-walled carbon nanotubes (SWCNT) etc. (**Sheng et.al 2011, Guo et.al. 2011, Cruz-Aguado and Penner 2008b**) are mostly utilized as quencher. For example, the fluorescence of carboxyfluorescein (FAM) is quenched readily via energy transfer from dye to carbon nanomaterials through "π– π" stacking interaction while FAM modified aptamer interacts with a carbon nanomaterial **(Zheng et.al. 2003, Zhu 2010).** Sheng et al. have reported that poly (vinyl pyrrolidone coated graphene-oxide) has significantly improved detection limit and sensitivity than uncoated graphene or SWCNT due to the control of the non-specific adsorption (**Guo et.al. 2011**).

In the series of fluorescence nanoparticles, up-conversion nanoparticles have brought a new avenue of simultaneous multi analyte detection. On the basis of doping with rare earth elements on UCNPs, varieties of multicolor finely tuned multiplexed-FRET donor-acceptor

pair can be developed with a combination UCNPs , doped with various rare earth elements like Er , Tm and Ho and graphene oxide having a wide range of absorption wavelengths (approximately 300−700 nm). Wu et al. have reported (**Wu et.al. 2010**) "signal on" aptasensor for multiplexed FRET-based turn-on assay for the simultaneous detection of OTA and fumonisin B1 (FB1). In another approach, it is demonstrated that N-(aminobutyl)-N-(ethylisoluminol) (ABEI), a luminol derivative, with AuNPs- modified electrode has been found to detect OTA (0.2-5.0 $ngmL^{-1}$) with a detection limit of 0.06 $ngmL^{-1}$ which is tenfold higher than the bare gold electrode (**Wang et.al. 2010**).

The nucleic acids, having ability to perform both as receptor and catalysis, are known as functional nucleic acids (FNAs) (**Li 2009**) or DNAzyme and hence, can be used for signal enhancement. Hemin as a ligand can specifically bind with several G-quadruplexes with high affinity and acts as a HRP-mimicking DNAzyme that may display a highly enhanced catalytic activity compared with hemin itself (**Cheng et.al. 2009, Li, et.al. 2009b**). Yang et.al. have designed a nucleic acid hairpin structure of hemin–G-quadruplex which opens up on addition of OTA. The activity of this DNAzyme is monitored spectrophotometrically via H_2O_2 mediated-oxidation with tetramethylbenzediene (TMB) as a colorimetric substrate (**Yang et.al. 2013).** Single, double, and triple HRP-DNAzymes coupled to the AFB1 aptamer have been tested and found signal amplification depends on number of the DNAzymes that produce chemiluminescence (CL) while binding to AFB1-ovalbumin (OVA) used as a coating antigen (**Shim et.al. 2014**)

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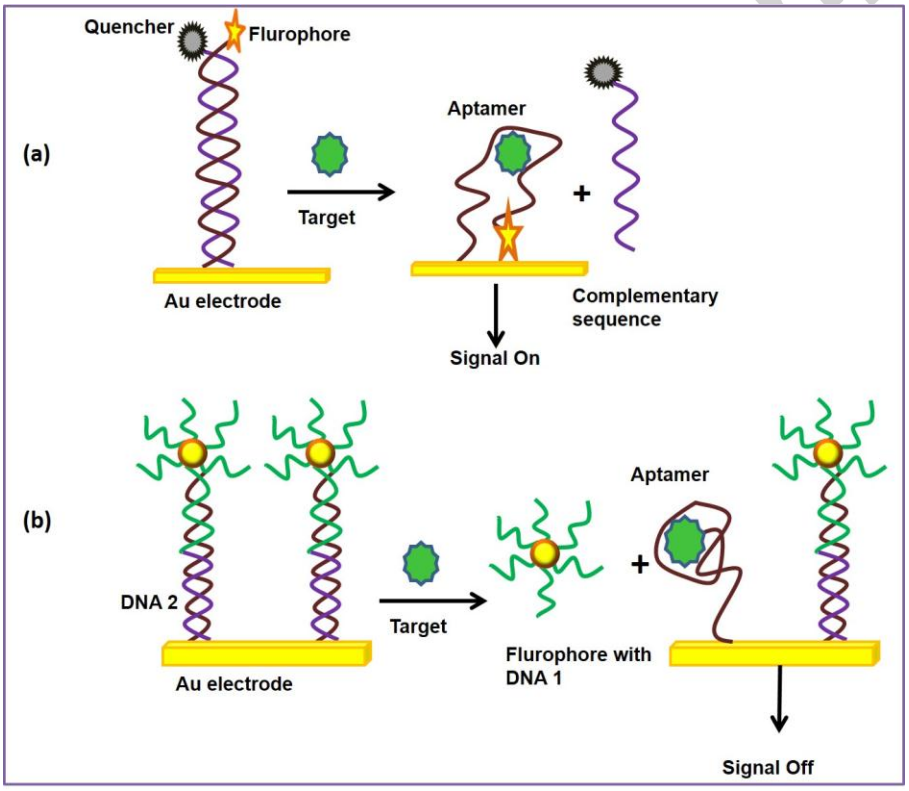

Fig.3 (a): Aptasensor 'signal on' phenomenon. (**b):** schematic representation of 'signal off' phenomenon based aptasensor

2.2.2.2. Advanced approach for signal amplification

The 1:1 binding ratio of the aptamer and target limits the signal enhancement and the sensitivity of the assay (**Tong et.al. 2011**). Signal amplification is an efficient way to improve the sensitivity of an aptasensor. Some signal amplification approaches such as polymerase chain reaction (PCR) (**Schmittgen and Livak 2008**), rolling circle amplification (RCA) (**Li et.al. 2010**), loop-mediated isothermal amplification (LAMP) (**Wang et.al. 2010, Fang et.al 2011**) and nicking endonuclease signal amplification (**Hun et.al. 2010**) have been reported. Chen et.al. have designed a sandwich nanostructured self-assembly of OTA binding aptamer and the DNAzymes on a gold electrode surface to quench the ECL emission of the dissolved O_2. On addition of OTA, the RecJf exonuclease leads to the target recycling disassembly of the nanostructures and "signal on" for significant ECL for OTA detection with the LOD of 75 $fgmL^{-1}$ (**Chen et.al. 2014**). The exploitation of the target recycling in the field of aptasensor is limited (**Tong et.al. 2012**). Therefore, for enhancing the labeling ratio with aptamer, Tong et.al. have applied RCA, an isothermal DNA replication technique, for nucleic acid amplification by phi29 DNA polymerase (see supplementary file **Fig.S2**) to detect OTA down to 0.2 $pgmL^{-1}$ the level

with a dynamic range spanning more than 4 orders of magnitude. The nicking endonuclease enzyme has attracted researchers' attention for circular amplification and design the sensitive analysis of targets (**Hun et.al. 2013**). The strategy based on combined cleavage of nicking endonuclease with target-induced strand release can be used to develop a bioassay for the detection of OTA.

2.2.2.3. Signal Off

In the signal off format, a significant reduction in signal due to formation of the target aptamer complex is monitored (**Fig. 3b**). The influence of non-specific adsorption of DNA to the final sensing results could be avoided by the "signal-off" detection model. Therefore, this "signal-off" sensor could potentially reduce the false positive results compared with the reported "turn-on" sensor. For example, the electrochemical signal of Au NP assisted methylene blue (MB), acting as a electrochemical redox probe, decreases significantly on adding OTA to the solution **(Kuang et.al. 2010**).

The use of magnetic bead functionalised aptamer fulfills two objectives: (i) non covalent immobilization by external magnetic field and (ii) sensing tool for OTA (**Rhouati et.al. 2013**). It may be noted that all above mentioned sensors require multiple steps which are not beneficial for the real applications. Wu et.al. (**Wu et.al. 2012**) have designed a one-step electrochemical aptasensor using the thiol- and methylene blue- (MB-) dual-labeled aptamer modified gold electrode for determination of OTA with a wide linear range from 0.1 $pgmL^{-1}$-1000 $pgmL^{-1}$ (see supplementary file **Fig.S3**) with the LOD of 0.095 $pgmL^{-1}$.

2.2.3. **Label-free aptasensor**

In a label free aptasensor, the transducer measures direct interaction of aptamer and analyte through the change in transducer signal (**Schoning & Poghossian 2002, Li et.al. 2011**).

The electrochemical aptasensors based on modified surface of electrode with polymer, metal nanoparticles and metal oxide (eg. TiO_2-chitosan, chitosan/polyaniline, and acacia gum) yields enhanced sensitivity for OTA detection (**Khan & Dhayal 2009, Khan et al. 2011**). Click chemistry helps immobilization and uniform distribution of aptamer over the electrode that may help in improving sensitivity and LOD (**Hayat et al. 2013, Ran et al. 2011)**. To develop label free sensor and overcome the background current, conformational change of aptamer upon target mycotoxin binding is monitored for the mycotoxin analysis. Hayat et al. have fabricated polyethylene glycol–aptamer conjugate to form two piece macromolecules. The macromolecules results in the formation of long tunnels on screen printed carbon electrode (SPCE) surface, while aptamer acts as gate of the tunnels for the detection of OTA (**Hayat et al. 2013**). The similar strategy has been followed to construct label free "signal on" aptasensor for OTA assay based on formation of G-quadruplex and release of two single strands, to bind with the Tb3+ ions resulting in enhanced fluorescence intensity (**Fig. 4**). The LOD have been noticed to be as 20pg/mL (**Zhang et al. 2013**).

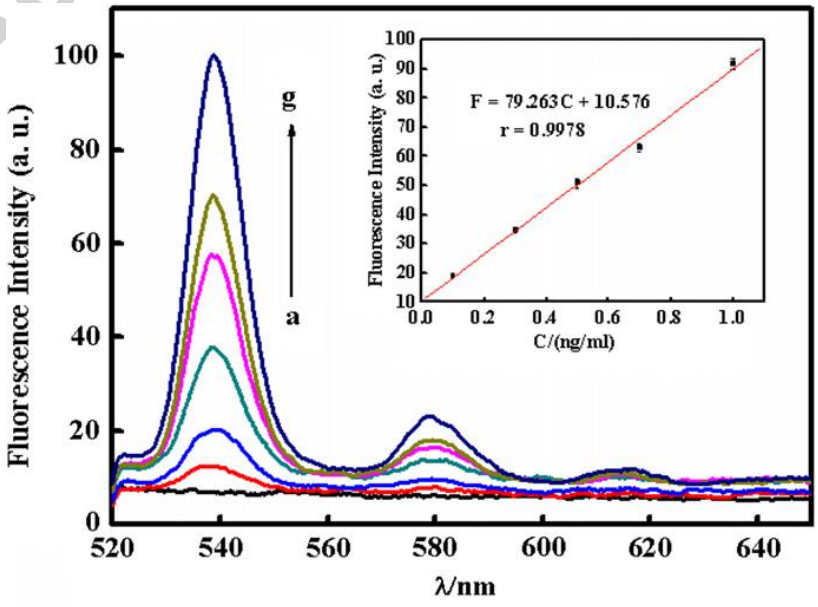

Fig. 4. Fluorescence spectra of the aptasensor with different concentrations of OTA (from a to g): 0.00, 0.05, 0.1, 0.3, 0.5, 0.7, and 1 ng/mL respectively. Inset: A calibration curve demonstrating peak fluorescence intensity versus OTA concentration (**Zhang et.al. 2013**).

For label free detection of small molecules, surface plasmon resonance spectroscopy is one of the highly sensitive technique. Hardly, any efforts have been made to utilize SPR spectroscopy in aptasensor for mycotoxins detection as that the change of SPR signal resulting from the adsorption on lower molecular weight aptamer is lower than that of antibody (**Wang et.al. 2009**). label-free aptasensor for OTA detection based on thickness shear mode acoustic method (TSM) (**Lamberti et.al. 2011**) and surface-enhanced Raman spectroscopy (SERS) (**Kim et.al. 2010, Galarreta et.al. 2011)** have also been reported**.**

In 2010 Neoventures Biotechnology Inc. (Canada) has patented specific aptamers to AFB1 and zearalenone. Firstly, Guo et.al. (**Guo 2014**) have fabricated a real-time quantitative polymerase chain reaction (RT-qPCR) based aptasensor for AFB1 detection. The RT-qPCR greatly improves detection sensitivity, and specificity of aptamer, functionalized at end 3′-with biotin groups. The sensing is accomplished in a single PCR tube with the detection limit of 25 $fgmL^{-1}$ for AFB1. The reported aptasensor for detection of mycotoxins are summarized in **Table 2.**

Table 2: Summery of labeled and label-free Aptasensor for the detection of Mycotoxins

S.N.	Matrix	Electrodes	Type of receptor	Mode	Label	Technique	Mycotoxin	Response Range	LOD	Sensitivity	Relative standard deviation(RSD)i	Coefficient of variations (CV) in %	References

											n %		
1	Magnetic Beads	-	Aptamer	Competitive	ALP	DPV	OTA	-	0.11 ng/mL	-	-	-	Barthelmebs et.al. 2011
2	Paramagnetic microparticle beads	SPCE	Aptamer	Competitive	HRP	DPV	OTA	0.78-8.74 ng/mL	0.07 ng/mL	-	-	-	Bonel et.al. 2011
3	SH-Apta	Au	Aptamer	Competitive	Fluorescein aptamer	Microfluidic channel SERS	OTA	0.05 to 4 µM.			-	-	Galarreta et.al. 2013
4	Magnetic beads based DNA (ssDNA) aptamers		DNA (ssDNA) aptamers	Non competitive	-	Fluorometer	ZON	3.14×10^{-9} to 3.14×10^{-5} M	7.85×10^{-10} M.	-	-	-	Chen et.al. 2013
5	6-mercapto-1-hexanol /captured probe(MCH)/(CP)/AuE	Au	aptamer	Competitive	-	Electrochemiluminescent	OTA	0.2 pg mL^{-1} to 1 ng mL^{-1}	75 fg mL^{-1}		9.1, 6.0	-	Chen et.al. 2014
6	Carboxyfluorescein	SWCNT	Aptamer	Competitive	-	Fluorescence	OTA	25 nM to 200 nM	24.1 nM		-	-	Guo et.al. 2011
7		magnetic bead (MB).	sing DNA		ABEI labeled carboxylic silica nano particles	chemiluminescence,	OTA	$1.0x10^{-12}$ to $5.0x10^{-8}$ g mL^{-1}	3.0×10^{-13} g mL^{-1}				Hun et.al. 2013
8	-	-		Competitive	Au NP - DNA	Electrochemical	OTA	0.1 to 20 ng/mL.	30 pg/mL,		-	10	Kuang et.al. 2010

9	-	-				Fluorescent	OTA	1 ng/mL-100 mg/mL	1 ng/mL		-	-	Lv et.al.2014
10	Magnetic beads	SPCE		Competitive	Biotin labeled OTA, ALP	Automated flow electrochemical aptasensor	OTA	1 µL to 3µL	0.05 µg/L		4-5	5	Rhouati et.al. 2013
11	Graphene oxide			Competitive	FAM (carboxyfluorescein)		OTA	2µM to 35µM.	21.8 nM		-	-	Sheng et.al. 2011
12	Graphene				UCNPs	FRET	OTA, FB1	0.05 to 100 ng·mL^{-1} for OTA and 0.1 to 500 ng·mL^{-1} for FB1	OTA 0.02 ng·mL^{-1} and FB1 0.1 ng·mL^{-1}.		4.74, 5.86	-	Wu et.al. 2012
13	Phi 29 DNA	Magnetic bead	Aptamer	-	QDs	Electrochemical	OTA	0.5 pg/mL-10 ng/mL	0.2 pg/mL	-	-	-	Tong et.al. 2012
14	Au NPs	Au	Aptamer	-	DNA 1- Au NPs DNA 2-ABEI	Electrochemi-luminescence	OTA	0.02-3 ng/mL	0.007 ng/mL	-	3.8	-	Wang et.al.2010
15	BSA or OVA	-	-	Competitive	Luminol or HRP	Chemiluminescence Aptamer	AFB1	0.1 – 10 ng/mL	0.11 ng/mL	-	-	-	Shim et.al. 2013
16	OTA chemisorbed on Au	-	Aptamer 4 sequences with one Dimer	-	-	EIS	OTA	0.1-100 nM	0.12-0.4nM	-	0.2-3.5	-	Castillo et.al. 2012
17		-	Apta	NC D	-	Fluoresce	OTA	-	5nM to 1	-	-	-	Cruz-Aguado

			mer			nce			pmol				et.al 2008
18		-	Aptamer	-	-	Spectro-fluorometer	OTA	1-100 ng/mL	0.8 ng/mL	-	4.7	-	Chan et.al 2012
19	16-mercapto-hexanedecanoic acid	-	Aptamer	D C	-	Thickness Shear Mode Acoustic Method (TSM)	OTA	25-740 nM	30 nM	-	-	-	Lamberti et.al 2011
20	Magnetic beads based DNA (ssDNA) aptamers		DNA (ssDNA) aptamers	Non competitive		Fluorometer	Zearalenone (ZEN)	3.14×10^{-9} to 3.14×10^{-5} M	7.85×10^{-10} M	-	-	-	Chen et.al 2013
21	solution		Carboxyfluorescein-OTA Aptamer	SWCNT		Fluorosence	OTA	25 nM to 200 nM	24.1 nM	-	-	-	Guo et.al 2011
22		magnetic bead (MB).	DNA		ABEI carboxylic silica nano particles	chemiluminescence (CL) probe,	OTA	1.0×10^{-12} to 5.0×10^{-8} g mL^{-1}	3.0×10^{-13} g mL^{-1}	-	3.4	-	Hun et.al 2013
23	streptavidin–biotin–DNA probe conjugates	chromatographic strip assay				scanning reader.	OTA		0.18 ng/mL	-	-	6.7	Wang et.al 2011
24									1.9 ng mL_1	-	4.7	-	Wang et.al 2010
25			aptamer			Electrochemical	OTA	0.1pg/mL – 1000pg/mL	0.095pg/mL	-	4.9	-	Wu et.al 2012
26	Tb3þ, structure-switchin				signal-on	Fluorescent	OTA		20 pg/mL OTA with high specificit	-	1.67	-	Zhang et.al 2013

	g aptamer and magnetic beads(MBs),a							y				
27	label-free for rapid and high-throughput detection of OchratoxinA		DNA zyme – aptamer conjugate			colorimetric	OTA	upto30nM	4nM	-	-	-	Yang et.al 2013
28	Ferrocene labeled DNA probe	Au	Aptamer	-	-	DPV	OTA	5pg/mL-10 ng/mL	1 pg/mL	-	-	-	Tong et.al 2011
29	Au NPs and DNA probe	Gold	ssHS DNA	Non-competitive	-	Impedimetric	AFM 1	1–14 ng/mL	-	-	-	6.2	Dinckaya et.al 2011
30	Biotin-Streptavidin	-	AFB1 Aptamer	Non-competitive	-	Electrochemical	AFB 1	5.0×10^{-5} to 5.0 ng mL^{-1}	25 fg/ mL	-	2	-	Guo et.al 2014
31	(Fe_3O_4/ PANI) film	Micro-IDE of Pt	21-mer aptamer	Non-competitive	-	Electrochemical based Aptasensor	AFM 1	6–60 $ng{\cdot}L^{-1}$,	1.98 $ng{\cdot}L^{-1}$.	-	5	-	Nguyen et.al 2013
32	11-mercaptoundecanol,Dextran 500,	Gold	-		-	DNA-based piezoelectric	AFB 1		1.5 ng/ g		-	10	Tombelli et.al 2009

2.3. Molecularly Imprinted Polymer (MIP)

Molecularly imprinted polymers (MIPs) are synthetic materials which can provide high affinity to specific target molecules. The self-assembly of functional monomers around the template molecule (target), followed by polymerization in the presence of excess cross-linkers to form a solid material. After removal of the template, cavities are created that are complementary in both shape and functionality to the original template. However, there are hardly any successful attempts have been reported for the development of MIP based mycotoxin analysis (**Piletsky et.al.2001**). Yu et al. have doped polypyrrole (PPy) with chloride (PPy-Cl) via electropolymerization of pyrrole on the gold surface of a miniaturized surface plasmon resonance (SPR) device for detecting OTA (**Yu and Lai, 2004, Yu and Lai, 2005).** A MIP electrochemical sensor has been recently constructed for the selective detection of T-2 by introducing iron ions (Fe^{3+}) to increase the chelation of the metal ions and templates **(Gao et al 2014).**

2.4. Enzymatic inhibition

An enzymatic inhibition analysis based on a variety of enzymes (cholinesterase, urease, glucose oxidase etc.) is commonly applied. Acetylcholinesterase (AChE) is the most frequently used enzyme due to it's sensitivity towards mycotoxins (**Puiu et.al. 2012**). Based on the inhibitory effect of AFB1 to AChE activity, biosensors for AFB1 detection is constructed using AChE (**Moscone et.al. 2011, Ben Rejeb et.al. 2009**). The enzymatic inhibition of AChE with AFB1 is a reversible binding and can real-time monitor mycotoxins through SPR etc. (**Puiu et.al. 2012**). Mycotoxins are reversible inhibitors that are non-covalently bound to the enzyme (**Stepurska et.al. 2015**). Out of a group of toxins, aflatoxin shows the maximum sensitivity of 0.015 μM (**Soldatkin et.al. 2013**).

2.5. Mimotope

Mycotoxins are toxic in nature, the necessity of pure toxin in synthesis of mycotoxin conjugates with protein molecules that may cause a toxicity risk to the manufacturers, users, and the environment. From a safety standpoint, it is advantageous to use a nontoxic chemical as an immunochemical reagent in place of toxic compound to enhance laboratory and environmental safety. And another disadvantage of using the conjugate is the release of analyte moiety from the conjugate that may give false signal to transducer. The mycotoxin conjugates can be replaced by mimotopes. Mimotopes are the peptides sequence of amino acids which show good affinity towards toxins, mimotopes selected from random phage displayed peptide libraries. Researchers have synthesized the mimotopes for various mycotoxins viz. OTA, ZEN, DON (**Bazin et.al. 2013, Lai et.al. 2009, He et.al. 2013**) and applied for the fast and accurate detection of mycotoxins.

3. Conclusions

Development of an appropriate biosensor for mycotoxin analysis in place of conventional chromatographic and ELISA technique is one of the thrust area in the field of biosensing research. The attempt of innovation is dedicated towards the choice of receptor (protein, nucleotides, MIP etc), strategy of transduction (electrochemical, optical, and EQCM), miniaturization (lab-on-chip, microfluidic etc), development of novel material (NPs, UCNPs), designing of signal generation (labeled and label free techniques) etc which lead to lowering of detection limit (up to the level of pg/mL), broad linear range and enhanced shelf life of the developed sensor etc.. In few cases, the researchers have applied the developed sensing system successfully on the contaminated food sample. No doubt, the research findings have shown the pathway of new science, novel technology, innovative material and state of art sensing technique etc but they are far away from the application in real life.

4. Future Trends

With the intervention of nano science and technology a new horizon has opened up in the field of device architecture. Researchers have paid their attention to exploit the advancement of technology for the construction of mycotoxin sensing tool. Unfortunately, till now the innovation is restricted in the laboratory scale, it has not reached to end user who badly needs a smart quick sensing reliable tool. Mycotoxin contamination is inhomogeneous in nature and demand extensive sampling to comply the legislation set by European Commission and other International agency. Carrying the exhaustive analysis using conventional tool such as chromatography with fluorescence (FLD) or mass (MS) detectors and ELISA technique requires time, cost, skilled man power and sophisticated instruments. Furthermore, these methods sometime may not reach to a very low limit of detection. In this context, biosensor is very promising for mycotoxin assay provided few challenges pertaining to technology and thought can be addressed judiciously such as (i)development of a synthetic receptor like aptamer, MIP etc in replace of costly unstable monoclonal antibody (ii) adaptation of label free easy scheme for detection and (iii)integration of modern technologies e.g, microfluidic and sensing design etc and (iv)finally a determined step for the conversion of innovation to application. Possibly future research can be directed to multi analyte detection on a lab-on chip platform with affordable cost.

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Highlights

- Immunosensor based on food mycotoxins detection
- Review focuses on the current research relating to development of immunoassays
- Immunoassay methods with emphasis on recent advances, challenges and trends.
- Present technological aspects in the development of immunosensors