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[INVITED] Novel optical biosensing technologies for detection of mycotoxins☆

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HIGHLIGHTS

- Total internal reflection ellipsometry (TIRE) was the main optical detection method.
- LSPR transducers were produced by annealing of thin gold films, then functionalized with half-antibodies or aptamers.
- The LSPR/TIRE combination allows the detection of mycotoxins in low concentrations (10 ppt).
- Optical planar waveguides is the way forward to development of highly sensitive and portable biosensors.
- Polarization interferometer sensor-prototype enables detection of mycotoxin in ppt concentrations.

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ABSTRACT

This work reviews our recent progress in development of novel optical methods of detection of mycotoxins in direct assay with either specific antibodies or aptamers. The main method in this work was the total internal reflection ellipsometry (TIRE) combined with LSPR transducers based on gold nano-structures produced by annealing of thin gold films. The gold nano-islands produced were characterised with SEM, AFM, UV–visible absorption spectroscopy, and spectroscopic ellipsometry. The combination of TIRE and LSPR offers superior refractive index sensitivity as compared to traditional UV–vis absorption spectroscopy. The limitations of LSPR related to a short evanescent field decay length can be overcome using small-size bio-receptors, such as half-antibodies and aptamers. The achieved sensitivity of detection of mycotoxins in 0.01 ppb level of concentration is sufficient for the use of this method for analysis of agriculture products, food and feed on the presence of mycotoxins. Even higher sensitivity in sub-ppt level was achieved with another optical biosensor developed recently; it is based on optical planar waveguide operating as polarization interferometer (PI). This method is promising for development of portable, highly sensitive, and simple to use biosensors suitable for point-of-need detection of mycotoxins.

1. Introduction

Mycotoxins are products of metabolism of numerous fungi species which can grow on different agriculture products, such as grains (corn, maize, rice, etc.), nuts, spices, coffee and cocoa beans, dried fruits, etc., at elevated temperatures and high humidity [1]. Therefore, mycotoxins appeared to be quite common contaminants in the above agriculture products and associated food and animal feed [1]. Furthermore, the presence of mycotoxins in animal feed causes their wider distribution in food chain, for example in poultries, meet, and milk [2]. Mycotoxins gained great deal of attention in the last 10–15 years because of their negative impact on human and animal health; many of mycotoxins are toxic, carcinogenic, and endocrine disruptive agents. The examples of three mycotoxins which were actually the subjects of study in this work are given below:

Aflatoxins B1 and M1 (AFT B1 & M1) [3] produced by *Aspergillus flavus* and *A. parasiticus* species grown on grains and cereals (maize, rice, wheat, etc.), spices (chilli and black pepper, coriander, turmeric,

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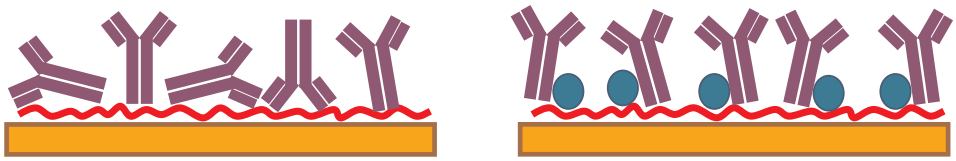

(a) (b)

Fig. 1. (a) Randomly oriented antibodies immobilized directly on polycation layer; (b) vertically oriented antibodies immobilized via intermediate layer of protein G (or A).

ginger), tree nuts (almond, pistachio, walnut, coconut, brazil nut); Aflatoxin B1 is one of the most carcinogenic substance known. AFT M1 being a 4-hydroxylated metabolite of AFT B1 is found in caw and sheep milk and milk products.

Ochratoxin A (OTA) [4] produced by *Aspergillus ochraceus, A. carbonarius*, and *Penicillium verrucosum* is one of the most abundant contaminant in grain and pork products, coffee, dried grapes, also in wine and beer, [5]. OTA is carcinogenic and neurotoxic for humans, and immunotoxic for animals.

Zearalenone (ZEN) [6] produced by *Fusarium or Giberella* species grown on crops (maize, barley, oats, wheat, rice, also bread) is a potent oestrogen metabolite causing infertility in swine and poultry.

The danger of mycotoxins was well-recognised worldwide, and recent legislations set quite strict limits for mycotoxins' content in food and feed [7]. The allowed dosage is slightly varied depending on the type of agriculture products, foods, and feeds. The lowest limits for mycotoxins in single ppb (part per billion) and even below (0.05 ppb for baby food) are established in EU, with similar standards in China and Japan, while US legislation is more lenient. More detailed information for particular products is available in [7]. The detection of small mycotoxin molecules (with molecular weight in 300–400 g/mol) in such small concentrations is a formidable task though not impossible. Modern analytical methods of mass-spectroscopy and chromatography are well-capable of detecting mycotoxins in ppt level of concentrations. However such advanced analytical methods are usually expensive and available in specialised laboratories; their use requires highly trained technical and academic personnel which makes the analysis very expensive and time consuming.

Much preferable solution would be portable and easy-to-use biosensor devices suitable for express, in-field detection of mycotoxins. The development of biosensors for mycotoxins has risen sharply in the last decade with a large number of different biosensing technologies been used which were extensively reviewed in [8–13]. According to review in optical biosensing of mycotoxins [10], the traditional SPR method allowed the detection of OTA down to 1.5 ng/ml in concentrations, while the use of LSPR in functionalized gold nanoparticles reduced LOD substantially down to 0.04 ng/ml. The SPR LODs for AFT B1 and ZON are, respectively, of 0.2 ng/ml and 0.3 ng/ml. Electrochemical detection of mycotoxins reviewed in [11] appeared to be more sensitive, for example LOD of 0.5 ng/ml for OTA detection with impedance spectroscopy; the use of aptamers in electrochemical detection of mycotoxins allowed decreasing LOD to 0.03–0.8 ng/ml for OTA, and 0.01 ng/ml for AFT B1.

Our contribution to development optical biosensors for mycotoxins was in the use of total internal reflection ellipsometry (TIRE) [14–17]. More detailed description of TIRE will be given in the following sections below. Briefly, this method is based on a combination of highly sensitive spectroscopic ellipsometry instrumentation with convenient Kretchmann SPR geometry. The TIRE method, which is based on optical phase detection, appeared to be much more sensitive than conventional SPR and thus extremely suitable for detection of low molecular weight analytes such as mycotoxins [15].

In this work, we developed the TIRE method further by combining it with the effect of LSPR (localized surface plasmon resonance). Detailed study of optical properties of gold nano-structures exhibiting LSPR phenomenon is given in this work followed by a series of bio-sensing tests of detection of several previously mentioned mycotoxins, e.g. aflatoxin, ochartoxin, and zearalenone. Another optical phase-sensing method of polarization interferometry based on planar optical waveguides is proposed here in a view of development of highly sensitive and portable (hand-held) optical biosensor devices suitable for in-field detection of mycotoxins.

2. Bio-receptors for detection of mycotoxins

Before going into details of optical techniques of TIRE, LSPR, and PI, which act as optical transducers converting bio-chemical processes into measurable optical parameters, it will be useful to discuss the biochemical aspects of detection of mycotoxins. The actual "detection" and "recognition" of our analytes of interest, e.g. mycotoxins, is achieved with the use of bio-receptors which are capable of their selective binding. The most common bio-receptors are antibodies which can be produced against particular targets, in our case mycotoxins. Bio-sensors based on the use of antibodies, usually called immunosensors, are the most common in optical biosensing. Antibodies are typically immobilized on the surface of optical transducers (metal films, optics fibers, nano-particles, etc.) using well-developed immobilization routes including covalent binding, electrostatic binding, encapsulation into polymer matrix, etc. Although, the strongest and thus the most stable covalent biding is commonly used in majority of optical immunosensors, in our work we used more simple method of electrostatic immobilization of antibodies which is the second strongest after covalent binding. A shown in Fig. 1, IgG type of antibodies being negatively charged at pH 7–8 can be electrostatically bound to a positively charged layer of polycations, such as poly-allylamine hydrochloride (PAH) or poly-ethylenimine (PEI) previously adsorbed on the transducer surface [18]; in this case IgG molecules are randomly oriented (Fig. 1a). Much better results (tripled sensitivity) can be obtained with the use of an intermediate body, i.e. proteins G or A (depending on the type of IgG used), which are electrostatically immobilized first on PAH (or PEI) layer followed by adsorption of IgG molecules having a binding site at the second domain to protein A (or G) [15]. In this case, IgG molecules are mostly oriented "vertically" with their Fab-fragments available for biding analyte molecules as shown in Fig. 1b.

The electrostatic immobilization of proteins including antibodies proved to be successful in our previous research [14–17], and we also used it in the current work. The immobilization procedure described previously in [14–18] was simple and consisted of consecutive immersions (injections) of PAH (1 mg/ml aqueous solution for 15–20 min, protein A (or G) (0.01 mg/ml solution in 35 mM Tris-HCl buffer, pH 7.5 for 15 min, and IgG-based antibodies (typically 1 μg/ml solution in Tris-HCl buffer pH 7.5 for 15 min.) with intermediate 3-times rinsing with de-ionized water (after PAH) and Tris/HCl buffer.

As shown schematically in Fig. 2, a simple splitting IgG-based antibodies by cutting di-sulphide bonds between two heavy chains through treatment with 2-mercaptoethylamine (stage 1) has resulted in two half-antibodies with thiol groups available for subsequent covalent binding on the surface of gold (stage 2) [19]. According to this study, the other di-sulphide bonds as well as fab-fragments in IgG are not affected. The half-antibodies for aflatoxin B1 were used in the current work; the immobilization protocol was similar to that described in [19].

Another possibility resulting in even smaller bio-receptors lies in the