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Detection of mycotoxins through nanobiosensors based on nanostructured materials: A review

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Abstract

Mycotoxins are secondary metabolites produced by fungi which can affect a variety of feedstuffs.These compounds elicit toxicological effects which represent risk for both humans and animals. Several sensitive methods for detection of mycotoxins based on chromatographic or immunochemical techniques are currently commercially available. The toxicity of mycotoxins occurs at very low concentrations, consequently there is a need of sensitive and reliable methods for their detection.The emerging nanotechnology has opened novel opportunities to explore analytical applications of the fabricated nano-sized materials. With the advent of nanotechnology and its impact on developing ultrasensitive devices, mycotoxins analysis is benefiting also from the advances taking place in applying nanomaterials in sensors development. During the last years, the highlight was put on nanoscale materials included in biosensors, which were some of the smart devices used for biomolecular detection. The using of nanoscale materials for biosensing systems has seen explosive increase in the recent years. The nanostructures such as nanoparticles, nanowires and nanorods could be considered as promising materials for construction of biosensors, facilitating the great improvement of the selectivity and sensitivity of the current methods. Implementation of nanomaterials in the fabrication of nanobiosensors and their use for the detection of mycotoxins in food and feed is the centre focus of interest of the current research work of many scientists.

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Introduction

The food safety and quality of the agricultural production requires of innovative and advanced technologies for contaminants monitoring. One of the most toxic contaminants is the mycotoxins. They are toxic secondary metabolites produced by organisms of the fungus commonly known as moulds.

It is important to note that they are toxic to animals and humans and for this reason these contaminants require detection in foods and feeds to maintain good food quality and to reduce their negative impact on the human health.

It should be mention that more than 500 different mycotoxins are currently known among which aflatoxins, ochratoxins, fumonisins, patulin and trichothecenes and they are considered important (Köppen *et al*., 2010). Contamination by mycotoxins is a real problem, not only because of economical losses, but especially because of the serious human and animal health problems that their ingestion may cause. Such problems include liver and kidney diseases, nervous system damage, immunosuppression and carcinogenicity (Turner *et al.,* 2009).

From another side mycotoxins are among the most common toxins in the agricultural sector. Generally they present in several food and feed, ranging in very limited range, and therefore there is a need of highly sensitive methods for their detection along the all food chain starting with the agricultural production (Tothil, 2011).

It should be noted that the currently used methods for mycotoxins detection have some disadvantages, i.e. time consuming, expensive, and less sensitive and no possibilities to detect very low concentrations.

For this reason there is a need of construction of more sensitive devices which will help their rapid detection. Taking into account the development of modern technologies the use of nanotechnology inspired systems will be powerful in delivering and fulfilling these requirements.

The impact of nanotechnology can be felt in agricultural systems for the improvement of crops, diagnostic of plant diseases, for monitoring the quality of agricultural products and as well as for detection of mycotoxins by nanobiosensors based on nanostructured materials. When we say biosensor, we mean an analytical device, which converts a biological response into an electrical signal. At least it consists of three parts: the sensitive biological element (e.g., an antibody, an enzyme, a protein, or a nucleic acid), the transducer and the associated electronics or signal processors that are primarily responsible for the display of the results, Fig.1.

The nanotechnology is not a single discipline or technology; it encompasses various disciplines, such as physics, chemistry, biology, electrical engineering, optical engineering and materials science. Typically this technology brings creativity and innovation to the investigation of the properties of materials when reduced to a very small size the so called ''nano'' size.
Because of dimensions smaller than 100 nm, the manipulation and creation of used nanostructured materials for detection of different small objects play an important role in the construction of biosensors. The development of biosensors fabrication technology is moving rapidly with new and novel nanomaterials recognition which can be applied as the sensing receptors for mycotoxins analysis. Biosensors and affinity sensor devices have been shown to have the ability to provide rapid, cost effective, specific and reliable quantitative and qualitative analysis (Tothil, 2001).

A large number of applications and versatility in nanobiosensors can make them choice of research. Nanomaterials possessing properties make them different from the conventional biosensors. It is important to note the applied nanomaterials may improve optical, mechanical, electrochemical or magnetic properties of biosensors. All these characteristics exhibited by nanobiosensors make them beneficial, with high safety in ensuring quality of food, its management and for risk assessment of mycotoxins contamination.

Various nanostructures have been investigated to determine their properties and possible applications in biosensors. These structures include nanotubes, nanofibers, nanorods, nanoparticles, thin films and etc., Fig. 2. The aim of the paper is to review the application of some nanostructured materials in the improvement of innovative and sensitive biosensing systems for mycotoxins detection.

Table1. Advantages and disadvantages of nanobiosensors and conventional methods for mycotoxins analysis.

Advantages		Disadvantages	
Conventional methods	*Nanobiosensors*	*Conventional methods*	*Nanobiosensors*
High resolution	Rapid analyses	Clean-up procedure	To increase the stability of some nanomaterials
Possibility to multiple detection system	Cost effective analyses	Specialist expert required	Reduce the aggregation of some nanomaterials
Suitable for rapid screening	Highly sensitive	Less sensitive Time consuming	Safe disposal after use because of toxicity to humans
Provides high level of conformation	Miniaturization, portability	High cost	Very sensitive and error prone
Number of samples can run together	Detect ultra-low concentrations of mycotoxins	Not able to detect very low concentrations	Still under infancy stage

Nanostructured materials

Nanoparticles

Among the most studied nanostructured materials are gold nanoparticles which have emerged as a promising material for biosensing that provide a useful complement to more traditional sensing techniques.

Nanoparticles are defined as small objects that behave as a whole unit in terms of its transport and properties. They have one dimension that is 100 nm or less in size. It is important to note that nanoparticles have numerous possible applications in biosensors construction. For example, functional nanoparticles bound to biological molecules (e.g. peptides, proteins, nucleic acids) have been developed for use in biosensors to detect and amplify various signals. The applications of nanoparticles have received increasing attention. Metal nanoparticles are generally defined as isolable particles between 1 and 50 nm size that are prevented from agglomerating by protecting shells (Bonnemann and Richards, 2001). For this purpose, gold nanoparticles have been extensively studied and biomimetic nanobiosensorsare developed for the detection of mycotoxins in food. Bio conjugates of enzyme and nanoparticles improve the electron transfer among the electrode and catalytic site of immobilized enzyme. This leads to enhance the biological stability of sensor surface and increases its analytical sensitivity.

In this connection Radoi *et al., (*2008) have established that the classical Enzyme-linked immunosorbent assay (ELISA) could be improved by decreasing the coating and competition time based on the use of super paramagnetic nanoparticles. Being super paramagnetic those nanoparticles are easily separated from the bulk solution, allowing also a versatile manipulation. This will allow ELISA to become faster and competitive. They have demonstrated that a competitive immunoassay for aflatoxin M1 based on the use of super paramagnetic nanoparticles was reliable, easy to perform and time efficient.

From another side Bonel *et al.,* (2010) showed that big advances in mycotoxin analysis are disposable biosensors capable of measuring *in situ* very low concentrations of ochratoxin A (OTA) with rapid and small instrumentation. They developed and compared the analytical properties of two indirect competitive immunosensors. Additionally the improvements of the nanostructured immunosensor with newly synthesized molecules of the antigen bound to gold nanoparticles are more sensitive EC_{50} = 0.68 ng/mL and measured lower limit of detection where (LOD) is 1.5 ng/ml of OTA. This is due to more accessible sites on the surface of electrode and increased electro catalysis current due to gold nanoparticles. These nanostructured immunosensors are capable of measuring of OTA below EU regulatory limits for cereals.

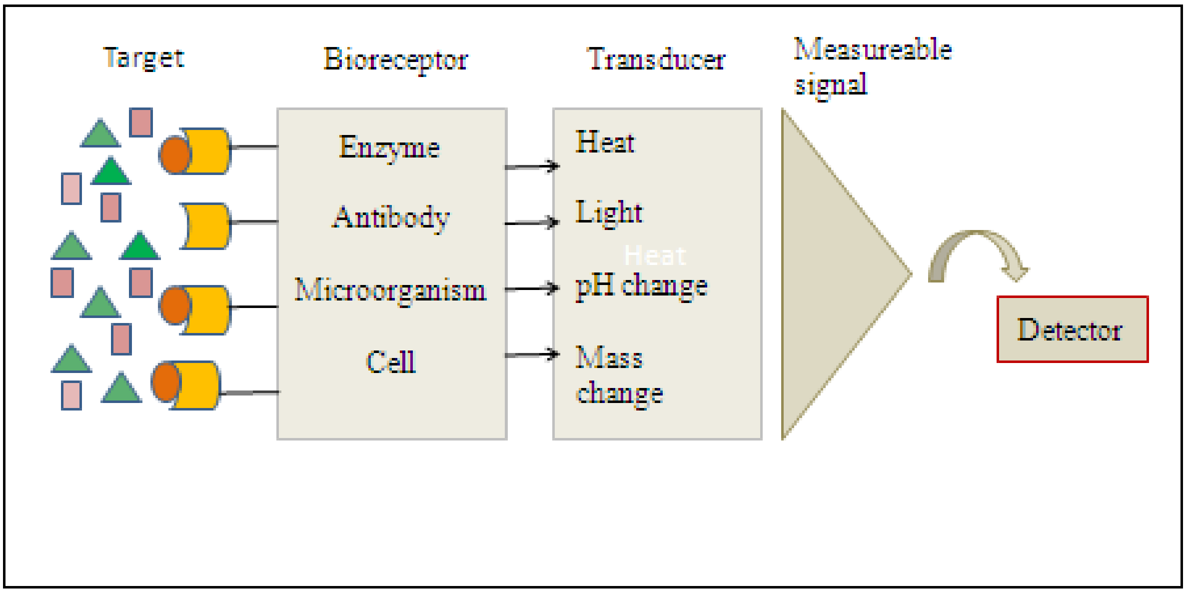

Fig. 1. Schematic representation of nanobiosensor.

For this purpose gold nanoparticles have been extensively used as matrices for the immobilization of macromolecules such as proteins, enzymes and antibodies as well as chemical labels for biomolecules. Another type of nanoparticles is magnetic nanoparticles. They are currently used in assays of biomedical and food-safety fields with the advantages of uniform diameters and even distribution in solution (Kuo *et al.,* 2012; Pappert *et al.,* 2012; Urusov *et al.,* 2014). Complexes between the nanoparticles and antibodies are formed by covalent immobilization. The immobilized particles can bind with the target antigens in solution and are rapidly separated by a magnetic field (Ohne *et al*., 2013; Smith *et al.,* 2012; Diler *et al.,* 2011). This technology has the advantages of liquid-phase immunological reactions, reduced detection time and improved sensitivity (Hu *et al.,* 2010).

Advancement in nanotechnology leads to the discovery of many methods useful for the detection and sensing of mycotoxins level in livestock (Shim *et al.,* 2009).

Moreover, the researchers are engaged in development of most sensitive techniques for the detection and management of mycotoxins and mycotoxigenic fungi (Jogee *et al.,* 2012).

In this connection it is interesting to mention that Gan *et al*., (2013) in their study have used magnetic nanoparticles for immobilization on graphene oxide and formed magnetic nanocomposites. These nanocomposites were then used as bioreceptor for adsorption of aflatoxins M1 biomolecules. Their results had proven the excellence of nanomaterials at bioreceptor level.

Nanomaterials when applied at transducer level, they may increases the electron transfer by behaving as an active quencher of electron and improves the signal amplification. Thus enhanced transducers activity. Holzinger *et al.* (2014) have given extensive review on how nanomaterials help to improve transducers functioning in nanobiosensors.

They have showed that the specific properties of the nano objects offer alternatives to classic transduction methods and that the combinations of different nanomaterials, each with its characteristics increase more the performance of biosensors is a well-accepted strategy.

In another study Fernandez-Baldo *et al.* (2011) used magnetic nanoparticles in preparation of nanobiosensors for detection of OTA. For this reason the nanoparticles were used as a platform to enhance the immobilization of biologically active constituents available in micro fluidic system for the fast and accurate sensation of OTA in apples. From another side to detect OTA in coffee Liu (2008) used gold nanoparticles for the synthesis of gold immune chromatographic strips.

It should be mentioned that recently, much research has been focused on the use of nanoparticles for various applications, which results in the development of different biosensor for the detection of different mycotoxins and to measure their level in the food commodity.

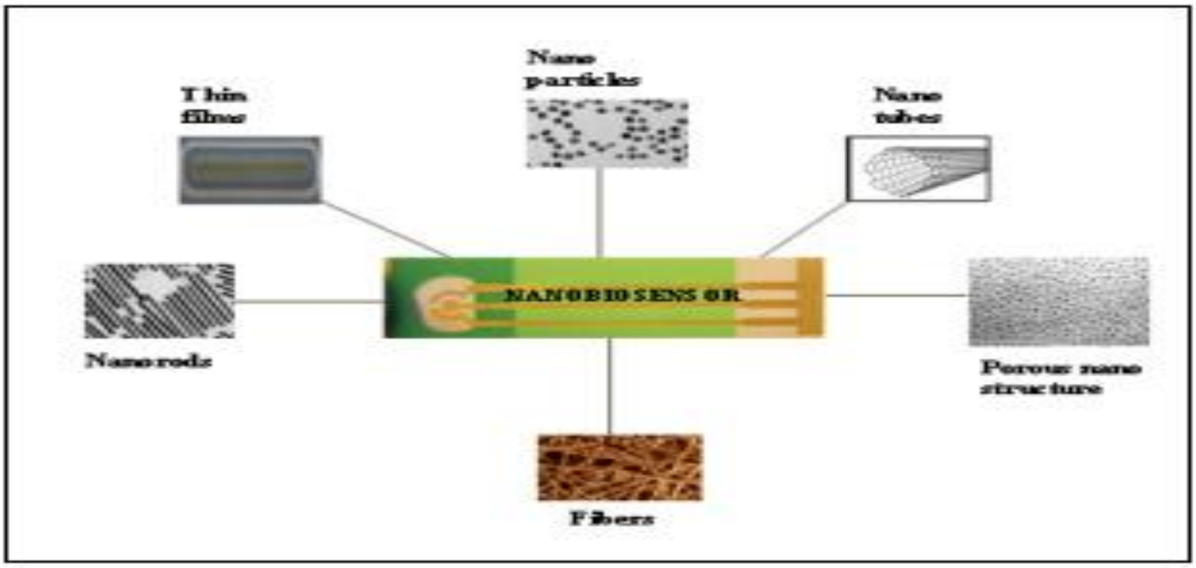

Fig. 2. Application of the most used nanostructured materials in nanobiosensors construction.

Surface Plasmon Resonance (SPR)

Another type of biosensors is based on SPR. And in this connection Gaag *et al.* (2003) developed biosensor on that principle for multiple mycotoxin analysis.

For this reason five types of specific antibodies were immobilized on the sensor surface to which five serially connected flow cells were attached, hence the analysis of five different mycotoxins at a time is possible.

The principle of SPR biosensor development is based on the binding of biological molecules on the thin metal film, which acts as a sensor. This results into changes in the mass concentrations, which are measured by the detector element. The free electron of the sensor metal plate absorbs light of specific wavelength at a specific angle of incidence, as a result intensity of the reflected light decreases. This phenomenon is dependent on the angle of incidence change because of changes in the mass concentration on the surface of the sensor. The activity of the sensor thus depends on the binding and dissociation of interacting molecules at the metal surface responsible for the mass concentration changes.

Ligler *et al.* (2003) reported on the use of biosensor consisting of a patterned array of capture antibody immobilized on planar waveguide. A fluorescent assay is then performed and the spots are captured using a CCD camera.

Several authors have reported the use of competitive immune fluorescent assays on a biosensor array for the simultaneous detection of different mycotoxins such as aflatoxin B1, fumonisin, OTA and deoxynivalenol (Ligler *et al.*, 2003; Sapford *et al.*, 2006).

A common use of tubular and other porous nanostructures in biosensors is to increase the quantity and activity of the immobilized biomolecules. However, in view of their unique properties, these nanostructures provide opportunities for development of novel designs of biosensors (Kumar *et al.*, 2014).

Cozzini *et al.* (2008) reported models for fast detection of mycotoxins on the basis of beta — cyclodextrins (β-CD) as mycotoxin receptors. They proposed a new efficient and cheap methodology based on a combination of computer chemistry, aided design and fluorescence. That could be used for synthesis in a more efficient way. The proposed new approach has been successfully applied to understand the different fluorescent behavior of aflatoxins B1 and OTA when complexed with β-CD in lack of structural information.

It has to be mentioned that following this model, the nanostructured polymer membrane could be used in construction of nanobiosensor where the pores of the membrane would act as receptors for fast mycotoxins detection on principle of host-guest system. For that reason the polymer membrane has to be processed with an appropriate surfactant to be ‘’able” to recognize the toxic metabolites (Sertova, 2015).

Carbon nanotubes (CNT's)

The nanobiosensors are supposed to be the second-generation biosensors, which are ultrasensitive due to involvement of nanomaterials in it. Traditionally used transducers in biosensors are now replaced by the use of nanoscale devices. In this connection Ajayan and Zhou (2001) have showed the unique behavior of carbon nanotubes (CNT) including their electrical, chemical, mechanical and structural properties. Moreover, CNT displays metallic, semiconducting and superconducting electron transport and they also possess a hollow core that can be used for storing a foreign molecule. They have become the subject of intense investigations since their discovery. Therefore the unique electrochemical properties of carbon nanotubes make them extremely attractive for electrochemical biosensors.

Depending on the number of concentric rolled sheets cylinders of which they consist, CNT can be divided into two general categories: single-walled (SWCNT—one rolled graphene sheet) or multiple-walled (MWCNT—two or more rolled graphene sheets).

Recent studies have demonstrated that CNT can be used to enhance the electrochemical reactivity of important biomolecules, and can be utilized to promote the electron-transfer reactions of proteins (including those where the redox center is embedded deep within the glycoprotein shell). In addition to enhanced electrochemicalreactivity, CNT-modified electrodes can be employed to accumulate important biomolecules (e.g., nucleic acids) and to alleviate surface fouling effects (Chen *et al.,* 2009).

CNT's exceptional properties (e.g., small size, great strength, high electrical, thermal conductivity, and large specific surface area) make them excellent amplification platforms to increase the number of signal-generating molecules. Mycotoxins are toxic fungal metabolites that can occur in primary food products such as nuts, cereals and fruits as a result of mould growth. Some mycotoxins have been proved as strong carcinogenic agents like aflatoxin B1; others are found to have carcinogenic effects. Currently a few hundred mycotoxins are known which are often produced by the genera Aspergillus, Penicillium and Fusarium. The most prominent toxins are aflatoxins, deoxynivalenol (DON), zearalenone, ochratoxin, fumonisin and patulin. Carbon nanotubes have been used in the development of electrochemical enzyme sensors and immunosensors due to their ability to promote electrontransfer reactions with electroactive species at low overpotentials. Moreover, their high surface-to-volume ratio provides a favourable environment to immobilized biomolecules (Malhotra *et al.,* 2015).

Singh *et al.* (2013) have fabricated carboxylated multiwalled carbon nanotubes based biosensor for aflatoxin detection. Pristine MWCNT has been synthesized by chemical vapour deposition. After that they are purified and functionalized through refluxing in concentrated nitricacid/sulphuric acid solution, generating a large number of COOH groups on the MWCNT surface. The carboxylated MWCNT that have been electrophoretically deposited onto indium tin oxide (ITO) substrate and the electrode surface was functionalized with monoclonal anti-aflatoxin B1 (anti-AFB1). The antibody molecules were covalently immobilized via EDC-NHS coupling chemistry to ensure the high stability. It has to be mentioned that authors found that the c-MWCNTs based electrochemical immune-biosensor showed high sensitivity (95µA ng-1mL cm-2), improved detection limit (0.25 ng mL-1) in the linear detection range of 0.25-1.25 ng mL-1.

Nanorods

Nanorods are also prospective nanostructured materials which could be incorporated in construction of nanobiosensor tools used for detection of mycotoxins.

The conventional optical biosensors were modified by the use of nanomaterials like gold nanorods (GNR) and proved better in the detection purpose for aflatoxins B1 at very low level of mycotoxins existence (0.04 ppb) in food commodity (Xu *et al.,* 2012). It is interesting to note that Xu*et al.* (2013) constructed an optical biosensor by using GNR dispersion on a competitive basis for detection of aflatoxins B1. Accordingly, as the concentration of aflatoxins B1 increases in solution, GNR dispersion also increases and absorption intensity of UV–visible spectra varies. The changes in the spectra and hydrodynamic size of GNR are recognized as indicator of sensors.

Concerning gold nanorods structure involved in nanobiosensors. Solanski *et al.* (2017) have reported the unique properties of nBi_2O_3nanostructurefabrication that make this material extremely interesting for biosensor applications. They showed that the electrophoretically derived nanostructured nBi_2O_3filmhas been used to fabricate an Ab-AFB1 based immunosensors for aflatoxin B1 detection.

It has been shown that the presence of nBi_2O_3 results in a favorable environment within creased electroactive surface. The fabricated BSA/Ab-AFB1/n Bi_2O_3/ITO immunoelectrode shows remarkably enhanced sensitivity and selectivity for aflatoxin B1. This immunosensor shows relatively rapid response (15 s), high sensitivity 1.132 µA/(ng/dL cm−2), broad linear range (1–70 ng/dL), low detection limit(8.715 ng/dL), linear regression coefficient 0.918, good reproducibility and long term stability.

The wide detection range and high sensitivity has been assigned to the amplification of the magnitude of current response since Bi_2O_3 nanoparticles with good biocompatibility maintain the biological activity of the antibody and improve the electron transfer between analyte (aflatoxin B1) and BSA/Ab-AFB1/n Bi_2O_3 /ITO immunoelectrode surface.

The low value of association constant (Ka) of0.234 nM indicates high antibody affinity of Ab-AFB1 to aflatoxin B1.The results clearly suggest that nBi_2O_3/ITO electrode provides an attractive matrix for effective immobilization of antibodies for fabricating of third generation biosensors and bioelectronics devices.

Becides of aflatoxins detection Wang *et al.* (2010) reported the procedure for the synthesis of nanobiosensors to detect OTA. They used gold electrode modified by GNP and N-(aminobutyl)-N-ethylisoluminol as label for specific OTA detection from contaminated wheat grains.

From another side Chen *et al.* (2009) have showed that the advantage of electrochemical nanobiosensor is the ability to detect mycotoxins at very low concentration up to 3 ng/mL.

In Table 1 are shown some of advantages and disadvantages of nanobiosensorsand conventional methods.

All these characteristics make nanobiosensors beneficial, with high safety in ensuring quality of feed and food. This kind of sensors based on micro and nano systems will become attractive tools for the researchers in the near future.

Conclusion

Nanobiosensors possessing properties make them different from the conventional biosensors. Applied nanostructures may improve optical, mechanical, electrochemical or magnetic properties of biosensors. Such modifications may enable them to detect mycotoxins at very low concentrations and to improve the quality of food and feed. Nowadays there is a big interest in the field of biosensors and especially in these ones constructed with nanomaterials. The use of nanostructured materials such as nanoparticles, nanorods and carbon nanotubes assay developments will enhance the capability and the sensitivity of the biosensor technology for mycotoxins analysis. Early detection will help to eliminating these toxins from entering the food chain. However, with the coming of nanotechnologies and their impact on developing ultrasensitive devices, mycotoxins analysis is benefiting from the advances taking place in applying nanomaterials in sensors development.

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