Application of nanotechnology in detection of mycotoxins and in agricultural sector

Prilozhenie na nanotehnologiite za otkrivane na mikotoksini i v agrarnija sector

Nadejda M. SERTOVA

Risk Assessment Centre, Bulgarian Food Safety Agency, 136, Blv. Tzar Boris III, 1618 Sofia, Bulgaria

E-mail address: sertova@hotmail.com *correspondence

Abstract

A brief review of nanotechnology application in detection of mycotoxins and in agriculture sector was presented. Mycotoxins are secondary metabolites produced by fungi. Their toxicity is the reason for implementation of various screening methods to detect them. During the last years, the highlight was put on nanoscale materials included in biosensors, which were some of the smart devices used for determination of mycotoxins, and in agriculture sector.

Over the next decade, the progress of nanotechnology will demonstrated a way to improve detection of contaminated feed and food. To achieve this purpose the innovations of nanomaterials reported every year would be applied. In the paper, some of the applications developed by nanotechnology that would contribute to the implementation of new tools for analysis of mycotoxins and agricultural products were discussed.

Keywords: agriculture, biosensors, mycotoxins, nanoparticles, nanotechnology

Резюме

Представен е кратък преглед за приложение на нанотехнологиите за откриване на микотоксини, както и в аграрния сектор. Микотоксините са вторични метаболити, произвеждани от гъбички. Тяхната токсичност е причина за прилагане на различни методи за откриването им. През последните години акцента е бил поставен върху нано материали, вградени в биосензори, които са част от модерните устройства, използвани за откриване на микотоксини, а също и в аграрния сектор. През следващото десетилетие ще бъде доказван напредъка на нанотехнологиите да бъдат използвани като метод за детекция на замърсени фуражи и храни. За постигането на тази цел всяка година ще се следят новостите при наноматериалите. В статията са обсъдени някои от приложенията,разработени с помощта на нанотехнологиите, които биха допринесли за изграждането на нови устройства за анализ на микотоксини и селскостопански продукти.

Ключови думи: биосензори, земеделие, микотоксини, нанотехнология, наночастици

Introduction

The last century was a good period for the progress of different technologies but nevertheless the accent was put on the nanotechnologies.
The word “nano” originates from the Greek word meaning “dwarf”. In the technical terms, the word “nano” means 10^{-9} meters.
Nanotechnology is the manipulation or self-assembly of individual atoms and molecules into structures to create materials and devices with new different properties. Materials with size in the range of 1 to 100 nanometres (nm) are called nanomaterials.
All organisms, from microbes to humans are powered by highly evolved molecular and cellular machines that operate at nano level. For example a leukocyte has the size of 10,000 nm, a bacteria 1,000-10,000 nm, a virus 75-100 nm and etc, Figure1.

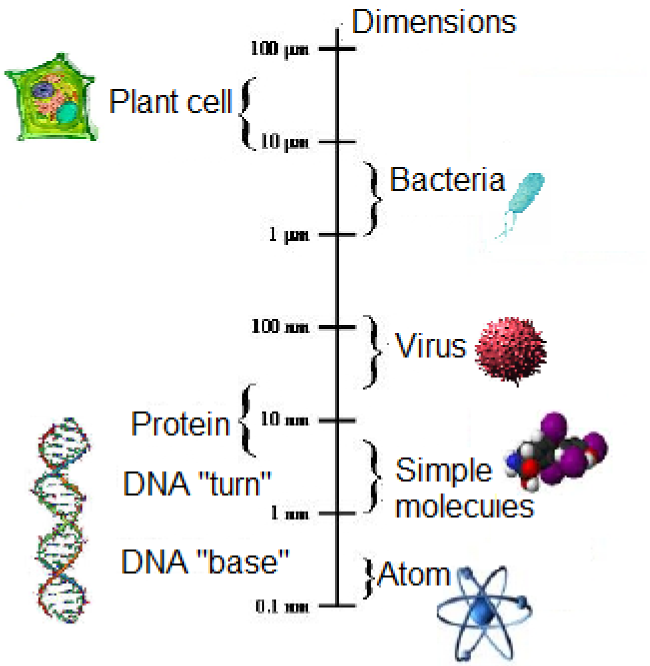


Figure 1. A scale to show the relative dimensions of various objects

And as a result of their size these materials should have different properties from those of the bulk material. These properties are connected with chemical reactivity and as well as with physical properties. Nature has been performing ‘’nanotechnological feats’’ for millions of years. Biological systems combine wet chemistry and electro-chemistry in a single living system (Prasanna and Hossain, 2006).
This convergence of technology with biology at the nano level is called ‘’nanobiotechnology’’. Nanobiotechnology is a new and exciting field of research. Recent advances in nanotechnology are integrated in the field of biology, in particular into molecular and cell biology.
Nanobiotechnology is an emerging area of opportunity that seeks to fuse nano/microfabrication and biosystems to the benefit of both. The impact of nanobiotechnology 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 using biosensors.

Role of nanomaterials in biosensors

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, Figure 2.

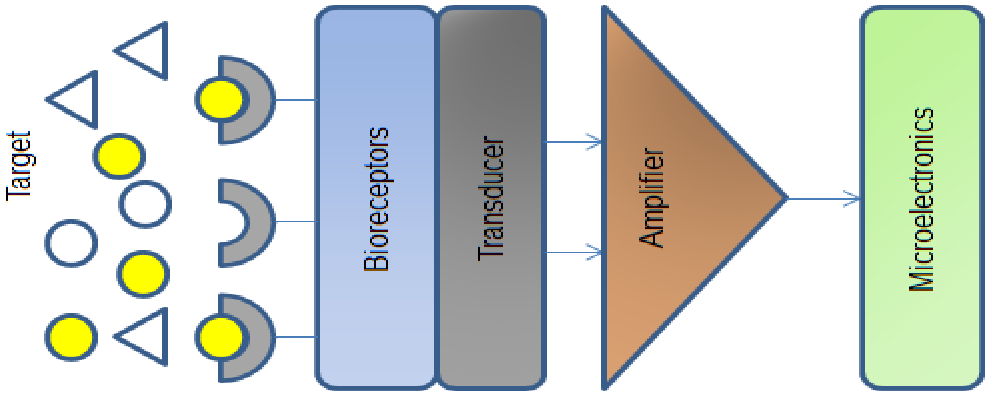


Figure 2. General schematic representation of biosensors

The biosensors can detect changes in cells and molecules at very low concentration of the tested material.
Therefore, if there is a large concentration of bacteria in a particular food, the biosensor will produce a strong signal indicating that the food is unsafe to eat (Center for Integrated Biotechnology, Washington State University).
It is important to mention that with this technology could be check the safety of food consumption.
Nanotechnology is playing an increasingly important role in the development of biosensors. The sensitivity and performance of biosensors could be improved by using nanomaterials for their construction. A wide variety of nanoscale materials are now available. The use of these nanomaterials allows the introduction of many new signal transduction technologies in biosensors. Because of their size nanosensors and other nanosystems are very important in the fields of chemical and biological analysis. Few interesting biosensor applications in food analysis are given in Table 1 (Viswanathan and Radecki, 2008).

Table 1. Applications of elelctrochemical biosensor for common analytes in foods

Analyte	Area of application
Organics:	
Amino acids, cholesterol, carbohydrates, pesticides, antibiotics, alcohols	Common constituents or contaminants in food products
L-alanine (with Balanine)	Flavor enhancer
Catechins, catechols and tannin	Taste and function of green tea. Quality control in tea processing (substances of astringency)
Polyphenols	Olive oils (taste and stability of the oil)
Acetaldehyde	Wine, beer, yoghurts
Malolactic acid	Wine quality
Inorganics:	
Sulphites, sulphur dioxide	Used as food preservatives, oxidation prevention
Potassium, sodium, calcium, magnesium, nitrate, nitrite, chloride, sulphate, fluoride, carbonate, and heavy metals	Vinegar, fruit juices, milk, soft drinks, mineral water

Applications of nanomaterial in biosensor for detection of mycotoxins

There are several applications of nanobiosensors in food analysis as well as in optical sensors used to detect pathogens and feed toxins. Thus, the light system in these biosensors is fluorescence, since this type of optical measurement can amplify the signal. Based on this property the nanobiosensors will be used for detection of mycotoxins. As it is known a mycotoxin is a toxic secondary metabolite produced by organisms of the fungus commonly known as moulds. These secondary metabolites are toxic to animals and humans. Generally, the mycotoxins present in several food and feed, ranging in very limited range, and therefore there is a need for highly sensitive and selective analytical methods for their detection. Nowadays there are different effectiveness systems working for detection of mycotoxins. One of them based on the properties of nanomaterials is included in the nanobiosensor devices.

The nanosensor’s structure includes materials at nano level and the most popular are nanoparticles. Nanoparticles are defined as small object that behaves as a whole unit in terms of its transport and properties. They have one dimension that is 100 nm or less in size.

Nanoparticles have numerous possible applications in biosensors. For example, functional nanoparticles bound to biological molecules (e.g. peptides, proteins, nucleic acids) have been develop to 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).

They can be used to enhance the quantity of immobilized biomolecules in construction of sensor (Viswanathan and Radecki, 2008).

On the other hand metallic nanoparticles, in particular gold nanoparticles (AuNPs), are nowadays of great interest in new generation of bioelectronic devices with increased sensitivity, high biocompatibility and novel functions (Wang, 2005).

The role of electrochemical nanobiosensor in feed and food analysis for detection of mycotoxins is an important and interesting area.

One of the most abundant food-contaminating mycotoxin is Ochratoxin A (OTA) (Ansari, et al., 2008; Pfohl-Leszkowicz, et al. 2002; Radi, et al., 2009; Zimmerli and Dick, 1996). It has found in tissues of organs of animals, including human blood and breast milk. OTA contaminations have been reported in cereals, coffee, wines, dried fruits and animal feeds (Kaushnik, et al., 2008; Zimmerli and Dick, 1996).

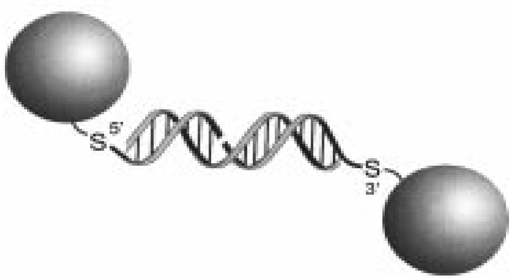
Instrumental methods for the analysis of OTA use mainly chromatographic based technique (Turner et al., 2009) mostly high pressure liquid chromatography (HPLC) assisted with molecular fluorescence detection (FLD) (Chiavaro et al., 2002; Shephard et al., 2003).

Chromatographic methods require multiple sample preparation steps prior to detection (Chung et al., 2007). Enzyme-linked immunosorbent assay (ELISA) is the most common immunoassay technique used in OTA analysis due to simplicity and capability for parallel analysis of multiple samples (Fujiil, et al., 2007; Monaci and Palmisano, 2004; Visconti and De Girolamo, 2005).

Even though more recently the use of biosensors allows a major portability, *in situ* analysis of OTA with similar selectivity and sensitivity to ELISAs and much shorter times of analysis (Goryacheva, et al., 2007; Parker and Tothill, 2009; Pohanka et al., 2007; Prieto-Simon et al., 2007).

L. Bonel et al. showed that big advances in mycotoxin analysis are disposable biosensors capable of measuring *in situ* very low concentrations of OTA with rapidly 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 (LOD) is 1.5 ng/ml of OTA). This is due to more accessible sites on the surface of electrode and increased electrocatalized current due to AuNPs. These nanostructured immunosensors are capable of measuring/determination of OTA below EU regulatory limits for cereals (Bonel, et al., 2010).

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. Sheme 1 shows a system based upon Au nanoparticles chemically modified with 5´- and 3´-(alkanethiol)-capped oligonucleotides. It is important to note that this system exhibits extraordinary selectivity and provides a simple means for colorimetric, one-pot detection of a target oligonucleotide in the presence of a mixture of oligonucleotides with sequences differing by one nucleotide, regardless of position, in the target region (Storhoff, et. al., 1998; Tang, et al., 2004; Xu and Han, 2004).



Sheme 1. Tail-to-tail alignment of gold nanoparticle probes

By modification of electrode surfaces with AuNPs provides a microenvironment similar to what obtains under physiological conditions (Liu, et al., 2003). Alternatively Joseph H. O. Owino et al. (Owino, et al., 2008) developed electrochemical immunosensor for detection of aflatoxin B1 (AFB1) by drop-coating of AuNPs on polythionine modified electrode surface.
They demonstrated that on the surface of the gold nanoparticles could be absorbed AFB1 conjugate.
This sensor exhibits high sensitivity and good reproducibility. These characteristics of the immunosensor show that it can be used to screen food products for AFB1.
The immunosensing procedure, which they reported in this study, eliminated the requirement of secondary labeled antibodies as it is in immunosensors based ELISA techniques.
As it is noted the most popular method for detection of mycotoxins contaminated in feeds is ELISA method.
More recently A. Radoi et al. established that the classical ELISA method could be improved by decreasing the coating and competition time based on the use of super paramagnetic nanoparticles. Being super paramagnetic these nanoparticles are easily separated from the bulk solution, allowing also a versatile manipulation. This will allow ELISA to become faster and competitive. They demonstrated that a competitive immunoassay for aflatoxin M1 (AFM1) based on the use of super paramagnetic nanoparticles was reliable, easy to perform and time efficient (Radoi, et al., 2008).
The versatility and high applicability of nanoparticles makes them clear candidates to be further used in nanosensors for feed analysis.
The most important here is that the biosensors are the binding and the specific analyte of interest to the sensor for the measurement with minimum of interference from other components in complex mixture.
The advantages and disadvantages of biosensors are essentially the same as those of immunoassays: they are cheap, fast, portable, very suitable for routine screening of samples, but they suffer from the selectivity and reproducibility problems (Cigic and Prosen, 2009). The results should be confirmed with the reference method

(Prieto-Simon, et al., 2007; Rodrigues-Mozas,et al., 2007). It was shown in the literature that some electrochemical biosensors showed excellent sensitivity with LODs below 0.1 μg/kg (Micheli, et al., 2005; Piermarini, et al., 2007) while those based on optical detection reached LODs comparable with ELISA (0.5-10 μg/kg) (Adanyi, et al., 2007; Prieto-Simon, et al., 2007). Although traditional immunoassay based methods such as ELISA, have been applied widely to mycotoxin detection. It should be noted that ELISA is still time consuming method. In this way the recent trend is to focus on immunosensors, which overcome the lengthy analysis time required by ELISA assays.

Among the immunoassay formats, non-competitive methods are the simplest and fastest although they are suitable for fluorescent compounds. Since the target fluorescence is directly detected the response of the analyte is directly proportional to the analyte concentration. Until now this approach has been used for AFB1 detection (Maragos and Thompson, 1999) but many mycotoxins have their own fluorescence (aflatoxins, OTA, zearalenon), and thus non-competitive assay could be potentially applied to multianalyte analyses.

In this way P. Cozzini et al. reported models for fast detection of mycotoxins on the basis of beta —cyclodectrins (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 AFB1 and OTA when complexed with β-CD in lack of structural information (Cozzini, et al., 2008).

Following this model, the pores of nanostructured polymer membranes could be used as receptors for fast mycotoxin 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.

In this connection Goryacheva et al. (Goryacheva, et al., 2008) investigated the fluorescent properties of aflatoxins in organized media and found that in the presence of surfactants the detection limit of the mycotoxins insignificantly changes in comparison with that one without surfactants.

Therefore, the pores of the polymer membrane would play a role as a cavity in β-CDs. Moreover, inside of the treated with surfactant pores the fluorescent groups could be protected for fluorescence detection. In particular, the natural fluorescence of the analyte can be use for a direct non-competitive assay, whereas a direct competitive assay requires the use of proper fluorescent label.

Figure 3 shows an image of nanostructured polymer membrane (Sertova, et al., 2009), which could be a prospective candidate for detection of mycotoxins and schematic picture of nanoporous membrane with analyte-diffusion.

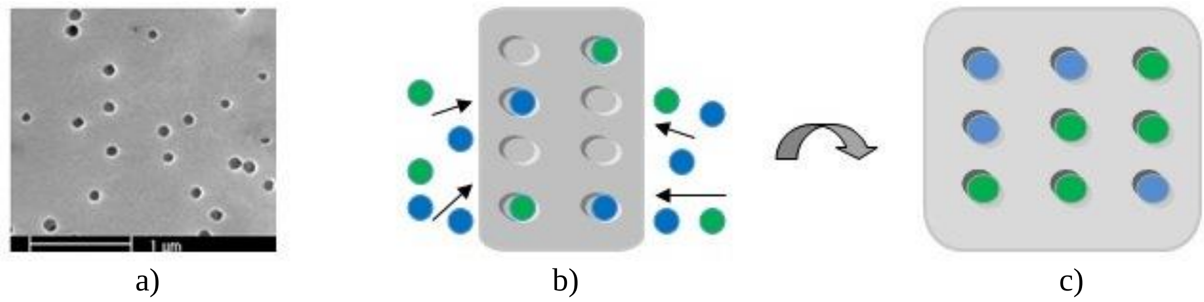


Figure 3. Nanoporous membranes: a) SEM image of nanostructured membrane; b) schematic depiction of the processed membrane embedded in the analyte; c) the membrane with the fixed inside fluorescent groups

In addition depending on the pore size such kind of nanoporous membranes can be selectively used for detection of different type mycotoxins, i.e. they can play a role as nano traps for toxins.

Application of nanotechnology in agricultural sector

In the last decade the nanotechnology touches on many fields by its scientific innovations. The agricultural and food industry are no exception. Coming nanotechnologies in agriculture look hopeful. The use of nanotechnology in agriculture has been mostly theoretical but it will continue to have a significant effect in some areas of the food industry: the development of new materials, biosensors for bio-security, in food packing in a way to ensure a better protection or to change the taste of food and as well as to ensure food safety.

As it was mentioned above the impact could be followed up in the field of crop improvement, where nanotechnology showed its flexibility to modify the genetic progress of crop plants thereby helping their further improvement of crop plants. Both mutations — natural and induced have played an important role in crop improvement (Prasanna and Hossain, 2006).

On the other hand, precision farming has been a long-desired goal to maximise crop yields while minimising fertilisers, pesticides, herbicides, etc. through monitoring environmental variables and applying targeted action. Precision farming makes use of computers, remote sensing devices to measure highly localised environmental conditions. In this type of farming the use of smart sensors will help the farmers to make better decisions. Although not fully implemented yet, tiny sensors and monitoring systems enabled by nanotechnology will have a large impact on future precision farming methodologies (Joseph, T., Morrison, M., 2006).

Other very important field, touched by nanotechnology, is diagnostic of plant diseases.

Diseases are one of the major factors limiting crop productivity. The viral diseases are the most difficult to control, as one has to stop the spread of the diseases by the vectors. Nevertheless, once it starts showing its symptoms pesticides application would not be of much use. The use of biomarkers could accurately indicate diseases stage. The nano-based diagnostic kits will increase the speed of detection and as well as the power too (Prasanna and Hossain, 2006).

Based on the unique properties of nanomaterials should be created an equipment of increased sensitivity, allowing an earlier response to the environmental changes. For example, nanoparticles or nanosurfaces can be prepared in a way to trigger an electrical or chemical signal in the presence of a contaminant such as bacteria. Nanoscale devices with unique properties could be used to make agricultural systems "smart" Such devices will identify plant health issues before these become visible to the farmer (Joseph, T., Morrison, M., 2006).

Nanomaterials can be introduced in food itself. The effectiveness of pesticides could be improved in a case if very small amounts are enclosed in nanocapsules, which can be designed to open only when triggered by the presence of the pest to be controlled. Nanopesticide residue on the food and from animal feed may end up inside the stomach but what happens then is not clear (Iranmania).

On the other hand, researchers are working on pesticides encapsulated in nanoparticles; these only release pesticide in an insect's stomach, which minimizes the contamination of plants themselves.

Another development of nanosensors is in food crops. The sensors will recognize when a plant needs nutrients or water, before the farmer to see any sign that the plant is deficient. The farmers then release fertilizer, nutrients, or water as needed, optimizing the growth of each plant in the field one by one. Really, it could be said for such sensors that they are one of the ''smart'' achievements of nanotechnology. Electronic or wireless network can be also attributed to the so called ''smart'' applications.

In agriculture the application of nanosensors is connected with their properties that they could be distributed throughout the field where they can monitor soil conditions and crop growth. They could be used for pest control of crops growing in the field. Particle farming is one such example, where the plants are growing in defined soils. For example, research has shown that alfalfa plants grown on gold rich soil absorb gold nanoparticles through their roots and accumulate these in their tissues. The gold nanoparticles can be mechanically separated from the plant tissue (Joseph, T., Morrison, M., 2006).

Agricultural and food area are going together since the nanotechnology applications are expected to be similar. Today the most important applications are biosensors. They are significant achievement of nanotechnology in these areas and they are used for analysis and detection of the quality of the agricultural products.

Additionally in the future bio and gas sensors could gain importance. These sensors could be integrated into packaging materials to monitor the freshness of food. Spoiling of food could be detected by a color change of the sensor (Prasanna, B., Hossain, F., 2006).

Manufactured nanomaterials are already used in some food products, nutritional supplements, and food storage applications.

The types of the storage applications include smart packaging, on demand preservatives, and interactive foods. The goal of some companies is to optimise product shelf life by implantation of smart packaging. Such packaging systems would be able to repair small holes, which should respond to environmental conditions (e.g.

temperature and moisture changes), and alert the customer if the food is contaminated (Iranmania).
Nanotechnology has the potential to revolutionize the agricultural and food industry with new tools for the molecular treatment of diseases, fast detection of diseases, enhancing the ability of plants to absorb nutrients etc., Table 2 shows some significant applications in the mentioned industries.

Table 2. Application of nanotechnology in agricultural and food industry

Agriculture	Food production	Food Packaging
Nanocapsules for delivery of pesticides	Nanocapsules for better taste	Nanogum and nanofilms for prevent oxidation and spoilage of products
Nanosensors for control of the soil	Nanoparticles for the selective removal of chemicals or pathogens from food	Robust and heat-resistant films with embedded silicate nanoparticles
Nanosensors for determining the pathogens in plants and animals	Nanoemulsions for better dispersion of food ingredients	Antibodies attached to fluorescent nanoparticles, which serve to trap chemicals in food

Smart delivery systems will help the agricultural industry to combat viruses and other crop pathogens. In the near future nanostructured catalysts will be available and will increase the efficiency of pesticides and herbicides, allow lower doses to use. Nanotechnology will also protect the environment indirectly using alternative energy supplies, and filters or catalysts to reduce pollution and clean-up existing pollutants.

Conclusion

This review shows the key aspects of nanotechnology applications for analysis of mycotoxins, highlighting current research in agriculture systems, as well as the future impacts they may have. It indicates the importance of the nanoparticles in the improvement of such classical method as ELISA to become easy in performance and time efficient in the process of the analysis.
Additionally the nanotechnology has a contribution in the construction of biosensors, which helps to be analysed very small quantities of different residue including mycotoxins.
In the agricultural sector, the nanotechnology promises to reduce pesticide use, improve plant and animal breeding, and create new nano-bioindustrial products. It also promises higher yields and lower input costs by streamlining agricultural management. The nanomaterials based on the developments including nanosensors to monitor the health of crops and farm animals.
On the other hand, a network of nano-sensors would relay detailed data about crops and soils. The sensors are able even to monitor the plant conditions. Nanoparticles or nanocapsules could provide more efficient means to distribute pesticide and fertilizers, reducing the quantities of chemicals released into the environment.

The reviewed nanoscale materials have already shown a good potential for being used in the agro food industry. Rapid testing of technologies and biosensor related to the control of pests and cross contamination of agricultural and food products will be certainly seeing applications of nanotechnology in the very near future.

In spite of all there is a potential risk arising from nanotechnologies on food and feed safety.

At present there is no reliable method of identification and control of nanoparticles in foodstuff and it should be keep in mind that the specific characteristic of nanomaterials could be connected with a potential health risk, namely the presence of potentially toxic nanoparticles in food.

Most likely in the near future the nanotechnology will change the food industry in such manner to make possible to predict the produced way, the processed way and as well as the packaged and transported way. Nowadays many countries have identified the potential of nanotechnology in the agro food sector and are investing a significant amount in it.

This will strengthen the adoption of nanotechnology in sensing applications, which will ensure food safety as well as alert the customers and shopkeepers when the shelf – life of food is expiring.

In the near future it is expected to be gave a ‘’green street’’ of the nanofabrication in most of the technologies. That promises for a modern style of life.

References

Adanyi, N., Levkovets, I. A., Rodriguez- Gil, S., Ronald, A., Varadi, M., Szdendro, I., (2007) Development of immunosensor based on OWLS technique for determining Aflatoxin B1 and Ochratoxin A. Biosensors and Biolectronics, 22 (6), 797-802. DOI:10.1016/j.bios.2006.02.015

Ansari, A., Kaushnik, A., Solanki, P., Malhotra, B., (2008) Sol–gel derived nanoporous cerium oxide film for application to cholesterol biosensor. Electrochememistry Communications, 10 (9), 1246-1249. http://dx.doi.org/10.1016/j.elecom.2008.06.003

Bonel, L., Vidal, J., Duato, P., Castillo, J., (2010) Ochratoxin A nanostructured electrochemical immunosensors based on polyclonal antibodies and gold nanoparticles coupled to the antigen. Analytical Methods, 2, 335- 341. DOI: 10.1039/B9AY00297A

Bonnemann, H., Richards, R., (2001) Nanoscopic metal particles — synthetic methods and potential applications. European Journal of Inorganic Chemistry, 2001, (10), 2455-2480. DOI: 10.1002/1099-0682(200109)

Center for Integrated Biotechnology. Washington State University, (2005), ’’The technologies & their applications’’, Available at: http://www.biotechnology.wsu.edu/definition_scope/tech&applications.html [21 April].

Chiavaro, E., Lepiani, A., Colla, F., Betonni, P., Pari, E., Spoti, E., (2002) Ochratoxin A determination in ham by immunoaffinity clean-up and a quick fluorometric method. Food Additives Contaminants., 19 (6), 575-581. DOI:10.1080/02652030210123869

Chung, Stephen, W.C. Kwong, K.P., (2007) Determination of Ochratoxin A at Parts-per-Trillion Levels in Cereal Products by Immunoaffinity Column Cleanup and High-Performance Liquid Chromatography/Mass Spectrometry, Journal of AOAC International, 90 (3), 773-777.

Cigic, I., Prosen, H., (2009) An overview of conventional and emerging analytical methods for the determination of mycotoxins. International Journal of Molecular Science, 10, 62-115. DOI: 10.3390/ijms10010062

Cozzini, P., Ingletto, G., Singh, R., Asta, C. D., (2008) Mycotoxin detection plays "Cops and robbers": Cyclodextrin chemosensors as specialized police? International Journal of Molecular Science, 9 (12), 2474-2494. Doi: 10.3390/ijms9122474

FujiiI, S., Ono, E. Y. S., Ribeiro, R. M. R., AssunçãoI, F. G. A., TakabayashiI, C. R., Oliveira, T. C. R. M., Itano, E. N., Ueno, Y., Kawamura, O., Hirooka, E. Y. (2007) A comparison between enzyme immunoassay and HPLC for ochratoxin A detection in green, roasted and instant coffee. Brazilian Archives of Biology and Technology, 50 (2), 349-359. Doi.org/10.1590/S1516-89132007000200020

Goryacheva, I., De Saeger, S., Nesterenko, I.S., Eremin, S.A., Van Peteghem, C., (2007) Rapid all-in-one three-step immunoassay for non-instrumental detection of ochratoxin A in high-coloured herbs and spices. Talanta, 72 (3), 1230-1234. DOI: 10.1016/j.talanta.2006.12.049

Goryacheva, I.Yu, Rusanova, T.Yu., Pankin, K.E., (2008) Fluorescent properties of aflatoxins in organized media based on surfactants, cyclodextrins, and calixresorcinarenes. Journal of Analytical Chemistry, 63 (8), 751-755. DOI: 10.1134/S1061934808080078

Iranmania, (2005) ''Iran Agro Sector developing nanotechnology'', Iranmania, Available at: http://www.iranmania.com/News/ArticleView/Default.asp?Newscode=35270&NewsKind=Current%20Affais. 7 September.

Joseph, T., Morrison, M., 2006 Nanotechnology in Agriculture Food. A Nanoforum Report, Available at: www.nanoforum.org, May.

Kaushnik, A., Solanski, P., Ansari, A., Ahmad, S., Malhorta, B., (2008) Chitosan –iron oxide nanobiocomposite based immunosensor for ochratoxin-A. Electrochememistry Communications, 10 (9), 1364-1368. http://dx.doi.org/10.1016/j.elecom.2008.07.007

Liu, S.., Leech, D., Ju, H., (2003) Application of colloidal gold in protein immobilization, electron transfer and biosensing. Analytical Letters, 36 (1), 1-17. DOI: 10.1081/AL-120017740

Maragos, C.M., Thompson, V.S., (1999) Fiber-optic immunosensor for mycotoxins. Natural Toxins, 7(6), 371-376. DOI: 10.1002/1522-7189(199911/12)

Micheli, L., Grecco, R., Badea, M., Moscone, D., Paleshi, G., (2005) An electrochemical immunosensor for aflatoxin M1 determination in milk using screen-printed electrodes. Biosensors and Biolectronics, 21(4), 588-596. DOI: 10.1016/j.bios.2004.12.017

Monaci, L., Palmisano, F., (2004) Determination of ochratoxin A in foods: state-of-the-art and analytical challenges, Analytical and Bioanalytical Chemistry, 378 (1), 96- 103. DOI 10.1007/s00216-003-2364-5

Owino, J., Arotiba, O., Hendricks, N., Songa, E., Jahed, N., Waryo, T. T., Ngece, R., Baker, P., Iwuoha, E., (2008) Electrochemical immunosensor based on polythionine/gold nanoparticles for the determination of Aflatoxin B1. Sensors, 8 (12), 8262-8274. DOI:10.3390/s8128262

Parker, C.O., Tothill, I.E., (2009) Development of an electrochemical immunosensor for aflatoxin M (1) in milk with focus on matrix interference. Biosensors and Bioelectronics, 24 (8), 2452-2457. http://dx.doi.org/10.1016/j.bios.2008.12.021

Pfohl-Leszkowicz A., Petkova-Bocharova T., Chernozemsky IN., Castegnaro M., (2002) Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. Food Additives and Contaminants, 19, (3), 282-302. DOI:10.1080/02652030110079815

Piermarini, S., Micheli, L., Ammida, N.H.S., Paleshi, G., Muscone, D., (2007) Electrochemical immunosensor array using a 96-well screen-printed microplate for aflatoxin B1 detection. Biosensors and Bioelectronics, 22 (7), 1434-1440. http://dx.doi.org/10.1016/j.bios.2006.06.029

Pohanka, M., Dun. J., Kuca, K., (2007) Mycotoxin assays using biosensor technology: a review. Drug and Chemical Toxicology, 30 (3), 253-261. Doi: 10.1080/01480540701375232

Prasanna, B., Hossain, F., (2006) Nanotechnology in agriculture. Pusa Agriscience, 29, 7-1.

Prieto-Simon, B., Noguer, T., Campas, M., (2007) Emerging biotools for assessment of mycotoxins in the past decade. Trends Anal. Chem. 26 (7), 689-702. DOI: 10.1016/j.trac.2007.05.012

Radi, A-E., Munoz- Berbel X., Lates, V., Marty, J-L., (2009) Label-free impedimetric immunosensor for sensitive detection of ochratoxin A. Biosensors and Bioelectronics, 15;24(7):1888-1892. DOI: 10.1016/j.bios.2008.09.021

Radoi, A., Targa, M., Prieto-Simon, B., Marty, J.-L., (2008) Enzyme-linked immunosorbent assay (ELISA) based on superparamagnetic nanoparticles for aflatoxin M1 detection. Talanta, 77 (1), 138-143. DOI.org/10.1016/j.talanta.2008.05.048

Rodrigues-Mozas, S., Lopez de Alda, M.J., Barcelo, D., (2007) Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography-mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. Journal of Chromatography A, 1152 (1-2), 97-115. Doi.org/10.1016/j.chroma.2007.01.046

Sertova, N., Balanzat, E., Trautmann, C., Toulemonde, M., (2009) Investigation of initial stage of chemical etching of ion tracks in polycarbonate. Nuclear Instruments and Methods B, 267 (6), 1039-1044. DOI: 10.1016/j.nimb.2009.02.045

Shephard, G., Fabiani, A., Stockenstrom, S., Mshicileli, N., Sewram, V., (2003) Quantitation of ochratoxin A in South African wines. Journal of Agricultural and Food Chemistry, 51(4), 1102-1106. DOI: 10.1021/jf0259866

Storhoff, J.J., Elghanian, R., Mucic, R.C., Markin, C.A., Lestinger, R.L., (1998) One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes. Journal of American Chemical Society, 120 (9), 1959-1964. DOI: 10.1021/ja972332i

Tang, D.P., Yuan, R., Chai, Y.Q., Dai, J.Y., Zhong, X., Liu, Y., (2004) A novel immunosensor based on immobilization of hepatitis B surface antibody on platinum electrode modified colloidal gold and polyvinyl butyral as matrices via electrochemical impedance spectroscopy. Bioelectrochemistry, 65 (1), 15-22. http://dx.doi.org/10.1016/j.bioelechem.2004.05.004

Turner, N., Subrahmanyam, S., Piletsky, S., (2009) Analytical methods for determination of mycotoxins: a review. Analytica Chimica Acta 632, 168 – 180. DOI: 10.1016/j.aca.2008.11.010

Visconti, A., De Girolamo, A., (2005) Review Fitness for purpose—ochratoxin A analytical developments. Food Additives Contaminants, 22, (1), 37- 44. DOI: 10.1080/02652030500410307

Viswanathan, S., Radecki, J., (2008) Nanomaterials in electrochemical biosensors for food analysis. Polish Journal of Food and Nutrition Sciences, 58 (2), 157-164.

Wang, J., (2005) Nanomaterial-Based Amplified Transduction of Biomolecular Interactions. Small, 1, (11), 1036-1043. DOI: 10.1002/smll.200500214

Xu, S., Han, X., (2004) A novel method to construct a third-generation biosensor: Self-assembling gold nanoparticles on thiol-functionalized poly (styrene-co-acrylic acid) nanospheres. Biosensors and Bioelectronics, 19 (9), 1117-1120. http://dx.doi.org/10.1016/j.bios.2003.09.007

Zimmerli, B., Dick, R., (1996) Ochratoxin A in table wine and grape juice: occurrence and risk assessment. Food Additives and Contaminants, 13(6), 655-668. DOI: 10.1080/02652039609374451