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Analytical, thermodynamical and kinetic characteristics of photoluminescence immunosensor for the determination of Ochratoxin A

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ABSTRACT

Ochratoxin A (OTA) is one of the most widespread and dangerous food contaminants. Therefore, rapid, label-free and precise detection of low OTA concentrations requires novel sensing elements with advanced bio-analytical properties. In the present paper we report photoluminescence (PL) based immunosensor for the detection of OTA. During the development of immunosensor photoluminescent ZnO nanorods (ZnO-NRs) were deposited on glass substrate. Then the ZnO-NRs were silanized and covalently modified by Protein-A (Glass/ZnO-NRs/Protein-A). The latest structure was modified by antibodies against OTA (Anti-OTA) in order to form OTA-selective layer (Glass/ZnO-NRs/Protein-A/Anti-OTA). In order to improve immunosensors selectivity the surface of Glass/ZnO-NRs/Protein-A/Anti-OTA was additionally blocked by BSA. Formed Glass/ZnO-NRs/Protein-A/BSA & Anti-OTA structures were integrated within portable fiber optic detection system, what is important for the development of low cost and portable immunosensors. The immunosensor has been tested in a wide range of OTA concentrations from 10^{-4} ng/ml until 20 ng/ml. Interaction isotherms were derived from analytical signals of immunosensor. Association constant and Gibbs free energy for the interaction of Glass/ZnO-NRs/Protein-A/Anti-OTA with OTA were calculated, analyzed and compared with some other related results. Sensitivity range and limit of detection were determined as 0.1–1 ng/ml and 10^{-2} ng/ml, respectively. Interaction kinetics of ZnO-NRs with OTA was evaluated. Response time of the immunosensor toward OTA was in the range of 500–800 s. Some insights related to the mechanism of PL-signal generation are proposed and discussed.

1. Introduction

The contamination of food by mycotoxins recently become very serious problem, because mycotoxins are very dangerous to human and animal health. Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus ochraceus*, *Aspergilius niger* and *Penicillium verrucosum*. OTA is found as a contaminant in a number of food products, such as cereals, coffee beans, beans, grapes and dried fruits. OTA is one of the most toxic and widespread compound from the ochratoxins group (Amézqueta et al., 2012; Gerez et al., 2014). Therefore, the detection of OTA and/or the determination of its concentrations is an important task in the control of food safety and quality. It has been reported that due to low concentrations of OTA in food and related products the analytical methods for OTA detection should satisfy requirements such as: high sensitivity, high specificity and simple operation principles. (Amézqueta et al., 2012; Gerez et al., 2014)

Different types of biosensors have been developed for OTA detection/determination (Ah et al., 2012; Bianco et al., 2017; Bougrini et al., 2016; Catanante et al., 2016; Wang et al., 2016; Zhang et al., 2016). Particularly, low concentration of OTA have been detected using impedimetric (Mejri-Omrani et al., 2016), capacitance (Bougrini et al., 2016), electrochemical (Catanante et al., 2016), field effect (Ah et al., 2012) and optical biosensors (Bianco et al., 2017; Wang et al., 2016; Zhang et al., 2016). Among the mentioned biosensors optical

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biosensors have demonstrated highest precision in the detection of OTA comparing to other types of biosensors. Some other recent approaches based on optical detection of OTA are relaying on surface plasmon resonance (SPR) and dye label fluorescence. Such methods showed good sensitivity to OTA in the range of 0.2–40 ng/ml (Bianco et al., 2017; Wang et al., 2016; Zhang et al., 2016).

Some insights on the application of immunosensors, which are based on interaction between specific antigen-antibody couple have been reported for the detection of OTA (Pagkali et al., 2017; Soares et al., 2016). Some developed biosensors showed high selectivity between OTA and aflotoxin B (Pagkali et al., 2017). ZnO-based nanostructures were shown as perspective semiconducting materials for the application in immunosensors (Shavanova et al., 2016; Tereshchenko et al., 2016). For instance, ZnO nanorods have relatively high isoelectric point (IEP) at pH=9.3 and efficient photoluminescence (PL) at room temperature in UV and visible ranges, which is important for the application in optical biosensors (Sodzel et al., 2015; Viter et al., 2016, 2014).

Previously we have reported immunosensor based on photoluminescence of ZnO-nanorods for the determination of *Salmonellosa* (Viter et al., 2014). Initially reported photoluminescent ZnO-nanorods-based biosensor (Viter et al., 2014) was improved by Sodzel et al. (Sodzel et al., 2015). Later Tereshchenko et al. (Tereshchenko et al., 2016), reported that ZnO-based nanostructures of different geometry (nanoparticles, nanowires, 1D nanostructures etc.) are very promising for PL-based biosensors due to higher concentration of surface states. Moreover, a surface of ZnO nanostructures could be easily modified by selective recognition exhibiting materials using various covalent and non-covalent immobilization methods (Tereshchenko et al., 2016). Thus, it can be predicted that 1D photoluminescent ZnO nanorods could be applied for the optical detection of OTA.

Compact size, low cost and reduced power consumption are the main requirements for portable sensor devices (Shavanova et al., 2016; Tereshchenko et al., 2016). Therefore, optical fiber technologies and laser emitting diodes could effectively substitute traditional detection techniques. For instance, a portable bio-analytic system, based on optical fiber technique and photoluminescent ZnO nanorods has been recently applied for the detection of cancer cells (Viter et al., 2016). Hence, the integration of this system with advanced biosensitive layers could make an impact on the development of portable biosensing systems (Viter et al., 2016).

In the present paper, we propose a fast and sensitive immunosensor for the determination of low concentrations of OTA by photoluminescence immunosensor based on ZnO-nanorods (ZnO-NRs). Low power PL-excitation source and optical fiber based technologies were applied to develop here described low cost, effective and portable OTA immunosensor.

2. Experimental

2.1. Materials

The biological samples (ochratoxin A (OTA)) and antibodies against ochratoxin A (Anti-OTA) (Ab), Protein-A, (3-Aminopropyl)triethoxysilane (APTES), bovine serum albumin (BSA) and glutharaldehyde were purchased from Sigma Aldrich.

2.2. Preparation and characterization of ZnO nanorods (NRs)

ZnO nanorod powder (ZnO-NRs) was synthesized as reported previously (Viter et al., 2016, 2014). Colloidal suspension of ZnO-NRs in butanol (concentration 1 mg/ml) was prepared by ultrasonication for 15 min. Glass substrates with dimensions of 1 × 1 cm^2 were prepared for the deposition of ZnO-NRs. Before the modification substrates were ultrasonically cleaned in ethanol and then they were treated by oxygen plasma to remove all organic compounds from the surface. Twenty microliters of the as-prepared ZnO-NRs solution were deposited on the cleaned glass substrate in order to form homogeneous ZnO-NRs layer on the glass surface. The samples were dried at room temperature and annealed in muffle furnace at 450 °C in air for 3 h. Scanning electron microscopy (SEM) imaging of the prepared samples was performed by Zeiss Evo HD15 SEM from Zeiss Ltd (Jena, Germany).

Optical properties of the ZnO-based nanorods were studied by photoluminescence (PL) spectroscopy. Nitrogen laser of λ=337 nm (LGI-21, Saint-Petersburg, Russia), with 0.4 mW output power was used as a PL-excitation source. The PL spectra were recorded by optical fiber spectrometer HR2000+, from Ocean Optics (Dunedin, USA) using 5 μm slit, 1 s integration time in the wave range of 360–420 nm.

Raman spectrometer Witek ALFA300R from Witek Ltd (Ulm, Germany) equipped with 532 nm excitation laser source was used for the registration of Raman spectra in the range of 50–2000 cm^{-1}.

ATR-FTIR spectrometer Tensor II from Bruker Optics was used to study interaction between the ZnO-NRs surface and immobilized biomolecules in the range of 400–3200 cm^{-1}.

2.3. Functionalization of the ZnO nanorods modified glass

In present work the ZnO nanorods were silanized according to the procedure described previously (Zhu et al., 2012). The glass slides with deposited ZnO-NRs were immersed in ethanol and dried in air. Then, the ZnO-NRs-modified glass slides (Glass/ZnO-NRs) were incubated in a chamber, containing 4% solution of APTES in toluene and the chamber was heated and kept at 70 °C for 1 h (Zhu et al., 2012). After the silanization samples were removed from the solution and rinsed with water and dried at 70 °C for 2 h. The APTES-modified Glass/ZnO-NRs structure was washed in phosphate buffer saline (PBS), pH 7.4. Then it was incubated in closed chamber containing vapor of glutaraldehyde formed over 10% glutaraldehyde solution in PBS, pH 7.4, at room temperature for 20 min.

In the next step, 5 μg/ml solution of Protein-A in PBS, pH 7.4, was deposited on the glutaraldehyde-activated surface of Glass/ZnO-NRs and incubated at room temperature for 30 min.

Antibodies against OTA (Anti-OTA) were deposited on Glass/ZnO-NRs/Protein-A surface from 6.7 μg/ml solution to form complex of Anti-OTA with Protein-A (Protein-A/Anti-OTA) and to form advanced structure of Glass/ZnO-NRs/Protein-A/Anti-OTA with oriented Anti-OTA antibodies (Makaraviciute et al., 2015). After the washing with PBS, pH 7.4, Glass/ZnO-NRs/Protein-A/Anti-OTA structure was incubated in 5 μg/ml solution of BSA in PBS, pH 7.4, for 20 min to decrease nonspecific interaction of other proteins with Glass/ZnO-NRs/Protein-A/BSA & Anti-OTA structure. The overall scheme of fabrication of layered Glass/ZnO-NRs/Protein-A/BSA & Anti-OTA structure on the ZnO-NRs surface is summarized in a Fig. 1.

The PL measurements were performed after each step of functionalization and interaction of formed Glass/ZnO-NRs/Protein-A/BSA & Anti-OTA structure with analyte. The parameters of PL measurements are described in Section 2.2.

Additionally, the immobilization and interaction of biomolecules was tested by ATR-FTIR spectroscopy. The PL and FTIR experiments are described in details in Section 2.2.

2.4. Immunosensor testing

The as-prepared Glass/ZnO-NRs/Protein-A/BSA & Anti-OTA structures were mounted in the experimental cell and 200 μL of PBS, pH 7.4, was injected. The immunosensor tests were performed after the stabilization of the PL signal.

OTA containing probes of 200 μL (of concentration varying from 0.0001 ng/ml until 10 ng/ml) were consequently injected into the immunosensor cell. Evolution of PL signal under OTA exposure has been measured until the signal reached steady-state conditions. OTA