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Visible-light driven label-free photoelectrochemical immunosensor based on TiO_2/S-$BiVO_4$@Ag_2S nanocomposites for sensitive detection OTA

Jinhui Feng[a], Yueyun Li[a,*], Zengqiang Gao[a], Hui Lv[a], Xiaobo Zhang[a], Dawei Fan[b,*], Qin Wei[b]

[a] School of Chemical Engineering, Shandong University of Technology, Zibo 255049, PR China
[b] Key Laboratory of Interfacial Reaction & Sensing Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China

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ABSTRACT

A label-free photoelectrochemical (PEC) platform with high visible-light activity for quantitative detection of the ochratoxin A (OTA) was developed by assembly of Ag_2S nanoparticles (NPs) sensitized on titanium dioxide/red blood cell-like shape bismuth vanadate (TiO_2/S-$BiVO_4$) electrode via layer-by-layer (LBL) strategy. In this protocol, ascorbic acid was used as an efficient electron donor for scavenging photo-generated holes and inhibiting light driven electron-hole pair recombination. TiO_2 has good photoelectric activity and large surface area. The S-$BiVO_4$ with porous structure surfaces can contribute to the high photocurrent intensity under visible-light irradiation. Moreover, the Ag_2S NPs were in-situ growth on surfaces of thioglycolic acid modified S-$BiVO_4$, which enhanced photocurrent response and further improved the photocurrent conversion efficiency. Under optimal conditions, the PEC immunosensor exhibited a wide linear concentration range from 5 pg mL^{-1} to 750 ng mL^{-1}, with a low detection limit of 1.7 pg mL^{-1} (S/N = 3) for OTA. Additionally, the designed immunosensor was performed with good stability, reproducibility and selectivity, thus opening up a new promising PEC platform for some other small molecules analysis.

1. Introduction

Ochratoxin A (OTA) is known as a powerful mycotoxin produced mainly by Aspergillus carbonarius and Penicillium verrucosum (Prabhakar et al., 2011; Zinedine and Mañes, 2009). It is one of the most toxic and cancerigenic substances and occurs widely in variety of mammalian species (Sorrenti et al., 2013). Moreover, it has been demonstrated that OTA has hepatotoxicity, nephrotoxicity, teratogenicity, and cytotoxicity, and can probably cause many kinds of tumor diseases in humans (Evtugyn et al., 2013; Lühe et al., 2003; Paterson and Lima, 2010). Therefore, early and accurately detection of OTA plays an important role to the health of human beings.

Recently, various methods have been applied to detect OTA, such as, high performance liquid chromatography connected to tandem mass spectrometry or fluorescence detection (Bazin et al., 2013; Brera et al., 2011; Zhao et al., 2014), surface plasmon resonance (Park et al., 2014) and enzyme linked immunosorbent assay (Alarcón et al., 2006; Liu et al., 2008). However, these methods required high cost, sophisticated instrumentation and complicated time-consuming, which hindered their wide actual applications (Wang et al., 2016; Yang et al., 2015). Thus, a highly sensitive method is necessary for quantitative detection of the OTA. With the fast developing of analysis technologies, the photoelectrochemical (PEC) assay has been widely applied in chemical analysis (Fan et al., 2015; Ma et al., 2015). In the PEC detection process, light is used as the excitation source for generating photocurrent used as the detection signal (Liu et al., 2016; Yin et al., 2016). Due to the effective differentiation between excitation light source and testing signal, the PEC sensor can significantly reduce the background signals and improve the sensitivity of detection. Moreover, PEC sensor presented many advantages including low manufacturing cost, short response time and excellent selectivity (Fan et al., 2014a; Shangguan et al., 2015; Yin et al., 2014). This work employs a label-free PEC immunosensor based on a strategy to achieve highly sensitive detection of the OTA.

Titanium dioxide (TiO_2), a semiconductor nanomaterial, has been proved to be an excellent basic material to develop PEC immunoassays because of its good photoelectric activity, large surface area, high stability and low cost (Li et al., 2012; Zhu et al., 2016). The $BiVO_4$ with red blood cell-like shape (S-$BiVO_4$) possessed porous structure surfaces has been improved in this study (Chen et al., 2016; Huang et al., 2014). The S-$BiVO_4$ was assembled onto the TiO_2 could promote the efficient transmission of electron and reduce the probability of photo-generated

* Corresponding authors.
E-mail addresses: liyueyun71@163.com (Y. Li), jndxfandawei@126.com (D. Fan).

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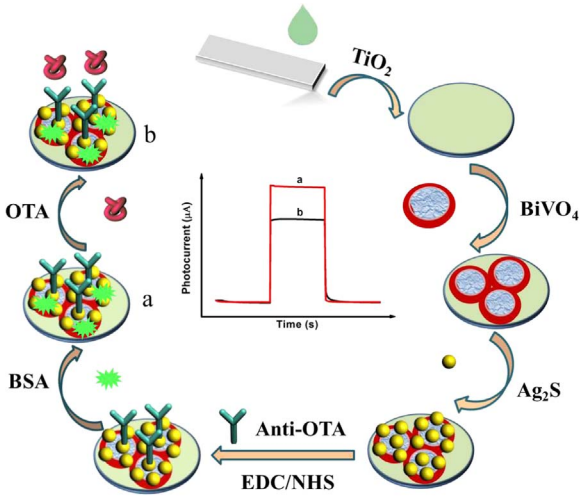
electron-hole (e^-/h^+) recombination. Recently, Ag_2S nanoparticles (NPs) have attracted many interest owing to their narrow band-gap (~ 1.0 eV), negligible toxicity, and relatively high absorb visible-light (Liu et al., 2015; Zhang et al., 2014). However, they exhibit low photocurrent conversion efficiency due to the e^-/h^+ recombination. The cascade band-edge levels can promote ultrafast transfer charge and effectively inhibited the e^-/h^+ pair recombination. Thus, the Ag_2S NPs were in-situ growth onto TiO_2/S-$BiVO_4$ via strong coordination interactions between sulfhydryl groups and $BiVO_4$ through thioglycolic acid (TGA), which enhance the photocurrent response in the visible-light region.

In this study, an ultrasensitive label-free PEC immunosensor based on Ag_2S sensitized on TiO_2/S-$BiVO_4$ with high photocurrent response was fabricated by layer-by-layer (LBL) method for the first time. The assembled strategy can not only adequately utilize the light energy but also effectively promote charge separation and consequently enhance the photocurrent conversion efficiency. The ascorbic acid (AA) served as an electron donor for scavenging photo-generated holes to suppress photo-generated e^-/h^+ recombination.



Scheme 1. Fabrication process of PEC immunosensor for OTA detection.

2. Experimental section

2.1. Materials and reagents

OTA and OTA antibody were purchased from Wanger Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma reagent Co., Ltd. (St. Louis, MO, USA). Indium-tin-oxide (ITO) glass (resistivity 10 Ω/sq) was obtained from Zhuhai Kaivo Electronic Components Co., Ltd. China. The other details are provided in Supplementary material (SM).

2.2. Apparatus

All PEC experiments were measured on a CHI760E electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd, China). A three-electrode system was used for all electrochemical measurements: a platinum wire electrode as counter electrode, a saturated calomel electrode as reference electrode and modified ITO electrode (2.5 × 1.0 cm^2) as the working electrode. A 100 W LED lamp (white light) was used as an irradiation source in the PEC test. The other details are displayed in SM.

2.3. Preparation of TiO_2 nanoparticles and S-$BiVO_4$

In this work, the TiO_2 NPs were synthesized according to the previous report with a slight modification (Huang et al., 2013). The S-$BiVO_4$ were prepared by self-assembled based on previous reports (Chen et al., 2016). The other details are provided in SM.

2.4. Fabrication of the label-free PEC immunosensor

Fabrication procedure of the proposed PEC immunosensor is displayed in Scheme 1. Prior to modification, ITO electrodes were ultrasonically cleaned in acetone, ethanol and ultrapure water for about 30 min, successively. After that, the ITO slices dried under the nitrogen stream for future use. Next, TiO_2 (5 mg mL^{-1}, 10 μL) was dropped onto an ITO electrode. After being dried, the S-$BiVO_4$ (3 mg mL^{-1}, 5 μL) was then dropped onto the above ITO/TiO_2 electrode. After being dried, the electrode was treated at 450 °C for 30 min in air and then cooled to the room temperature. The deposition of Ag_2S on TiO_2/S-$BiVO_4$ surface was according to the successive ionic layer adsorption and reaction with some modification (Wang et al., 2015): The TGA (0.1 mol L^{-1}, 3 μL) solution was dropped onto the ITO/TiO_2/S-$BiVO_4$ electrode surface and dried under the infrared lamp about 30 min; After washing with ultrapure water, $AgNO_3$ solution (0.1 mol L^{-1}, 3 μL) was dropped onto above electrode surface and reacted for 30 min under the dark, then washed with ultrapure water to remove uncombined of $AgNO_3$; The Na_2S solution (0.12 mol L^{-1}, 3 μL) was dropped onto the resulting electrode for reaction about 30 min to make Ag_2S full growth at the room temperature, then washed with ultrapure water to remove excess Ag_2S thoroughly. Finally, the desired ITO/TiO_2/S-$BiVO_4$@Ag_2S electrode was obtained.

The TGA (3 mmol L^{-1}, 3 μL) solution was dropped onto the ITO/TiO_2/S-$BiVO_4$@Ag_2S electrode and dried at room temperature. After washing with ultrapure water, 4 μL of EDC/NHS solution containing 1 × 10^{-2} mol L^{-1} of EDC and 2 × 10^{-3} mol L^{-1} of NHS was dropped on terminal carboxylic acid groups of the ITO/TiO_2/S-$BiVO_4$@Ag_2S/TGA electrode to activate –COOH for 1 h at room temperature, followed by washing with ultrapure water to wash off the excess EDC/NHS. After that, the anti-OTA (10 μg mL^{-1}, 4 μL) was immobilized onto the above resulting electrode via the classic EDC coupling reaction between carbonyl groups on the surface of the TGA-covered Ag_2S and amino groups of the anti-OTA and dried at 4 °C (Fan et al., 2014b). Then the electrode was washed to remove the loosely bounded anti-OTA. Subsequently, BSA (1 wt%, 3 μL) was incubated on the modified electrodes and further drying at 4 °C to block non-specific binding sites (Li et al., 2017c; Wang et al., 2017a), followed by washing with buffer solution thoroughly. Finally, different concentrations of OTA were dropped onto the electrodes respectively and then incubated at 4 °C, followed by washing with buffer solution to remove unbound OTA. After rinsing, the ITO/TiO_2/S-$BiVO_4$@Ag_2S/TGA/(EDC/NHS)/anti-OTA/BSA/OTA electrodes were fabricated successfully and stored at 4 °C for further PEC measurements.

2.5. PEC detection

PEC detection was carried out in PBS (pH = 7.4, 1/15 mol L^{-1}) containing 0.1 mol L^{-1} AA at room temperature. The AA was served as sacrificial electron donor during the photocurrent measurement. Photocurrent was measured by the current-time curve on a PEC workstation. A 100 W LED lamp (white light) was used as an irradiation source in the PEC test and was switched on and off every 10 s. The applied potential was 0 V.

2.6. Detection mechanism

The photo-generated e^-/h^+ transfer mechanism of the PEC immunosensor in the environment of ascorbic acid (AA) electrolyte was shown in Scheme 2. For TiO_2/S-$BiVO_4$@Ag_2S assembled structure, the TiO_2 substrate, S-$BiVO_4$ and Ag_2S possessed different optimal absorption bands due to different energy gaps, which adequately utilized the