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Analytical Methods

Highly sensitive microcantilever-based immunosensor for the detection of carbofuran in soil and vegetable samples

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ABSTRACT

Microcantilever-based immunosensor is a next-generation electromechanical technique with broad application in biological detection. In this paper, we reported a microcantilever-based immunosensor that quantitatively detect the carbofuran, by using monoclonal antibodies to carbofuran as the receptor molecules. The surface of gold-coated microcantilever was chemically modified by the crosslinking of L-cysteine (L-cys)/glutaraldehyde (GA). The monoclonal antibodies to carbofuran were then immobilized on the side of the microcantilever to fabricate the immunosensor, the mechanical bending induced by antigen-antibody specific binding under an experimental environment. Under the optimized conditions, immunosensor detected carbofuran showed a good linear relationship over the range from 1.0×10^{-7} to 1.0×10^{-3} g/L (R = 0.998), with a detection limit of 0.1 ng/mL. Moreover, the proposed immunosensor exhibited high sensitivity, specificity and good stability and can be successfully applied in the carbofuran determination in soil and vegetable samples with satisfactory results.

1. Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranol N-methylcarbamate, Fig. 1A) is one of the carbamate compounds and is used as a broad spectrum insecticide that kills insects, mites and nematodes in agricultural crops and plants (Tennakoon, Karunarathna, & Udugampala, 2013). Carbofuran can shorten the crop growth period and increase the crop yields, it has been widely used in the field of agriculture. However, owing to its high toxicity, weak degradability and it is high solubility in fresh water at 0.7 g/L, which can be absorbed by roots, stems and leaves, and then transported to various organs of the plant (Tan et al., 2015; Wang, Huang, Wang, Zhang, & Chen, 2014). The maximum residue Limit (MRLs) for carbofuran has been established by different legislations in many countries, for instance, in the case of rice, MRLs is 0.2 mg/kg in China and 0.1 mg/kg in Japan (Chen, Zhao, Liu, Zhou, & Yang, 2009). Additionally, carbofuran can cause acute oral toxicity to humans and animals (rat LD50, 8–14 mg/kg) through the potent cholinesterase inhibition (Nelson, MacKinnon, Mower, & Wong, 1981). Therefore, it is essential to establish a simple and sensitive monitoring method that strengthen the surveillance of carbofuran in agricultural and environmental samples.

Conventional methods, including voltammetry (Rao, Loo, Sarada, Terashima, & Fujishima, 2002), spectrophotometry (Jan, Shah, & Khan, 2003), amperometry (Sales et al., 2008), fluorimetry (Li, Zhang, Zhao, & Hu, 2010; Wong & Sotomayor, 2014), gas–chromatography (GC) (Rossi et al., 2001), high-performance liquid chromatography (HPLC) (Chen et al., 2009; Nogueira, Sandra, & Sandra, 2004; Vera-Avila et al., 2012), gas chromatographic–mass spectrometric (GC–MS) (Petropoulou, Gikas, Tsarbopoulos, & Siskos, 2006) and enzyme-linked immunosorbent assay (ELISA) (Yang et al., 2008) have been widely used for reliable and sensitive detection to carbofuran. While these operations are either expensive, time-consuming or complex (Sun, Zhu, & Wang, 2012). For these reasons, there is a growing demand for a more economical and simple method of detecting carbofuran. Furthermore, the proposed method could be used to detect carbofuran without any need for labeling with fluorescent or radioactive molecules.

In recent years, biosensors have drawn the interest of researchers due to their advantages such as simplicity, high sensitivity, lower cost and short analysis time (Cosnier & Holzinger, 2011; Grawe et al., 2015; Jin, Xu, Yu, Mao, & Hu, 2016). It is a device for the detection of analyte that based on a specific and sensitive biological recognition element in combination with a transducer,

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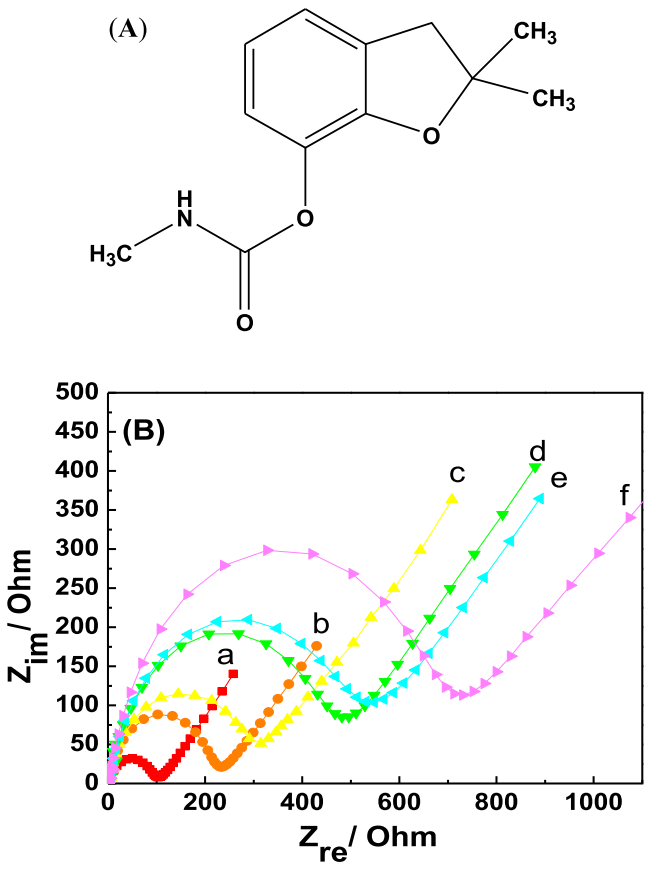

Fig. 1. (A) Chemical structure of carbofuran. (B) Nyquist plot (Z_{im} vs. Z_{re}) for Faradaic impedance measurements in the presence of 0.01 M PBS containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M KNO_3 at: (a) the bare Au electrode, (b) L-cys/Au, (c) GA/L-cys/Au, (d) carbofuran antibody/GA/L-cys/Au, (e) gelation/carbofuran antibody/GA/L-cys/Au and (f) carbofuran/gelation/carbofuran/antibody/GA/L-cys/Au.

with a physicochemical detector component for signal processing. Our research group managed to produce an electrochemical impedance spectroscopy (EIS)-based sensor for the detection of carbofuran and got a good result (Liu et al., 2015). The analysis principle of this work based on the formation of antibody-antigen complexes and it could reduce electron transfer rate between the electrode and an electrically active probe molecule, resulting in a further increase in the electron-transfer resistance (Rct). The difference of electron-transfer resistance before and after immunization was measured to achieve the sensing of target analytes. Biosensors being expected to have great application prospects in such wide-ranging areas such as health care, medical diagnostics, agricultural monitoring, food safety, environmental screening and the detection of harmful substances for military application (Ciriello, Cataldi, Crispo, & Guerrieri, 2015; Shan, Mousty, & Cosnier, 2004; Velusamy, Arshak, Korostynska, Oliwa, & Adley, 2010).

A microcantilever is an extreme, sensitive transducer and responds to the detection of target molecules in nanoscale units (Alvarez & Lechuga, 2010; Fritz et al., 2000). It consists of a tiny horizontal beam, when one side of the cantilever is coated with specific sensing molecules, the microcantilever immunosensor can recognize a corresponding analyte. Deflection length of the microcantilever can be measure by the electrical and optical-based detection. Piezoresistivity of a material under external strain has been converted into monitor the deflection of the microcantilever sensors (Khemthongcharoen et al., 2015). Meanwhile, it is inexpensive and portable. However, it needs to overcome the choice of material and the design of sensor. The optical detection based on the atomic force microscope (AFM) and with higher sensitivity. Additionally, the multiplexed microcantilever biosensor (an array of cantilevers), individually functionalized with different sensing layers, biochemical multi-analysts can be simultaneously detected in multi-channel system (Alodhayb, Rahman, Rahman, Georghiou, & Beaulieu, 2016). More recent uses of microcantilever-based biosensors have been applied to the measurements of DNA hybridization, viruses, enzymes, small-molecular and drug testing (Xue et al., 2011; Hou et al., 2013; Kim et al., 2015; Wang, Morton, et al., 2014). In previous work, we reported microcantilever-based sensor which functioned with L-cys and GA for highly sensitive detection of avian influenza virus, with a detection limit of 1.9 ng/mL (Xu et al., 2014).

Herein, our research group proposed a label-free microcantilever-based immunosensor for the analysis of carbofuran for the first time. It was constructed by modifying L-cys and GA on the surface of gold-coated microcantilever, using monoclonal antibodies to carbofuran as the receptor molecules. The antigen and antibody binding on the surface of the microcantilever gave rise to a tensile stress which caused microcantilever deflection. The tensile stress was induced by antibody conformational change (Wu et al., 2013). Measuring the degree of microcantilever deflection, the quantitative analysis method of antigen concentration was determined. The proposed microcantilever-based immunosensor could detect carbofuran in the range of 1.0×10^{-7}–1.0×10^{-3} g/L and exhibit high selectivity, good stability. In addition, it was successfully applied in the determination of carbofuran in soil and vegetable samples with satisfactory results. The non-competitive enzyme-linked immunosorbent assay (ELISA) was employed for verifying the application of the proposed method.

2. Experimental

2.1. Materials

Carbofuran antibody and antigen were provided by the College of Environmental Science and Engineering in Yangzhou University. L-Cys and gelatin aqueous solution (0.5%) were purchased from the Shanghai Chemical Reagent Company. GA was obtained from the Sinopharm Chemical Reagent Company. Goat anti-rabbit IgG, horseradish peroxidase (HRP) were purchased from Sigma-Aldrich (A6154, USA). All other chemicals were of analytical grade. All experimental solutions were prepared with deionized water (18.2 MΩ at 25 °C) obtained from a Millipore Milli-Q system. The phosphate buffer solution (PBS, pH 7.0, 20 °C) was prepared by mixing 0.02 mol/L disodium hydrogen phosphate solution and 0.02 mol/L sodium dihydrogen phosphate solution. The preparation of carbofuran standard solution (containing 10% methanol): carbofuran was dissolved with methanol and then diluted with PBS (pH 7.0, 20 °C). The solubility of carbofuran in methanol was better than that in water. 10% methanol as solvent, it could improve the solubility. It also can be easily removed by heating or vacuum distillation after completing the reaction.

2.2. Apparatus

All responses of the present immunosensor were recorded by using an atomic force microscope (AFM, Digital Instruments, Multi-mode Nanoscope III (a) scanning probe microscope, USA) equipped with a diode laser, a spatial filtering and focusing system, and an in-house-built position-sensitive optical detector. The flow cell is an accessory of this AFM and made up of the silica glass. The micro-fabricated cantilever is 193 μm in length, 20 μm in width,