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An ultrasensitive competitive immunosensor using silica nanoparticles as an enzyme carrier for simultaneous impedimetric detection of tetrabromobisphenol A bis(2-hydroxyethyl) ether and tetrabromobisphenol A mono(hydroxyethyl) ether

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ABSTRACT

Based on our produced polyclonal antibody capable of recognizing tetrabromobisphenol A bis(2-hydroxyethyl) ether (TBBPA-DHEE) and tetrabromobisphenol A mono(hydroxyethyl) ether (TBBPA-MHEE) (cross-reactivity, 100% for TBBPA DHEE; 98.7% for TBBPA MHEE), an important derivative and byproduct of tetrabromobisphenol A (TBBPA), respectively, a novel ultrasensitive competitive immunosensor was established using an electrochemical impedimetric strategy for the simultaneous detection of both chemicals. A significantly amplified electrochemical impedance spectroscopy (EIS) for quantitative target analysis was obtained through (i) the biocatalytic precipitation of 4-chloro-1-naphthol (CN) on the electrode surface triggered by horseradish peroxidase (HRP) and (ii) increased amounts of the enzyme with HRP-loaded silica nanoparticles carrying polybrushes (SiO_2@PAA) as labels, achieving a remarkable improvement in catalytic performance. Under the optimized conditions, the immunosensor showed satisfactory accuracy (recovery, 84.6–118%) and a good linear range (0.21- 111.31 ng/mL) with a limit of detection (LOD) of 0.08 ng/mL (S/N = 3) for TBBPA DHEE and TBBPA MHEE. In addition, the proposed approach was used to analyse real environmental water samples, and our results indicated that this immunosensor had great potential for the determination of the trace pollutants in aquatic environments.

1. Introduction

Tetrabromobisphenol A (TBBPA) is regarded as one of the most important brominated flame retardants (BFRs) with the highest production volume in the world, representing approximately 60% of the total BFRs market (Law et al., 2006; Liu et al., 2016a). Approximately 18% of TBBPA was used to generate derivatives (Covaci et al., 2011), and most of them were applied as an additive or reactive flame retardant in polystyrene foams, polyolefin resins, engineer polymers and epoxy resins (Covaci et al., 2009). However, increasing concerns on their potential environmental and health risks were expressed because the chemicals could be released into the environments after extensive use (Gu et al., 2017; Liu et al., 2016a). Among the derivatives, tetrabromobisphenol A bis(2-hydroxyethyl) ether (TBBPA-DHEE) should be further investigated due to its potential neurotoxins and the highest toxicity compared to the structure-related compounds (Liu et al., 2016b). So it is necessary to develop a sensitive and effective method to investigate the environmental occurrence of the organic containment for risk assessment.

To date, only two instrument analytical methods and one immunoassay were reported for the detection of TBBPA-DHEE and tetrabromobisphenol A mono (hydroxyethyl) ether (TBBPA-MHEE, one of the byproducts of TBBPA). Liu developed a sensitive method to detect TBBPA-DHEE and other major TBBPA derivatives with a high-performance liquid chromatography coupled with inductively coupled plasma tandem mass spectrometry, and its limit of detection (LOD) for TBBPA DHEE could reach 0.14 ng/mL (Liu et al., 2017). Using an ultra-high-performance liquid chromatography-Orbitrap Fusion Tribrid mass spectrometer, Liu also presented an approach for the determination of TBBPA-MHEE (Liu et al., 2015a). Given that these methods have the

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advantage of high sensitivity in quantification and identification, they suffer from expensive equipment, cumbersome pre-treatment procedure and the large volume of sample required in measurement (Zhang et al., 2010, 2017b). To solve the problem, we proposed a high-throughput indirect competitive enzyme-linked immunosorbent assay (ELISA) for monitoring both the chemicals using our previously produced antibody that possess almost equal cross-reactivity for TBBPA-DHEE and TBBPA-MHEE (Zhang et al., 2017a). However, the shortages of the conventional ELISA (limited sensitivity, labour-intensiveness and time-consuming manipulations) hinder further application of this technique in some cases (Chen et al., 2015; Liang et al., 2017). In contrast, impedimetric immunosensors are becoming increasingly attractive because of its simplicity, portability, ultra-sensitivity after various amplifying techniques (Cecchetto et al., 2015; Hou et al., 2016; Janegitz et al., 2017; Peng et al., 2016), therefore, it can be used as a more suitable method to investigate the trace pollutants in the environments.

The fabrication of an electrochemical immunosensor is usually based on the antigen-antibody interactions with electrochemical transducers (Fang et al., 2014), and the bio-interface with the immobilized bimolecular species on the electrode surface plays an important role in this method (Arkan et al., 2014). Benefited from the advanced development of nanomaterials, a hybrid structure of multi-wall carbon nanotubes/graphene oxide nanoribbons (MWCNTs/GONRs) was proved to be successful as its applications in this system (Liang et al., 2017; Liu et al., 2015b), which contributed to improve the dense immobilization and long-term stability of the attached antibody/antigen. Furthermore, signal amplification can be performed with enzymatic reactions to adjust the steric-hindrance and electrostatic-repulsion effect (Akter et al., 2012); this is especially important for a strategy based on enzymatic biocatalytic precipitation, building polymeric layers (insoluble products) on the electrode surface to block the electron transfer process of a redox probe, bettering the sensitivity (Akter et al., 2012, 2015; Hou et al., 2013; Tang et al., 2017; Wan et al., 2015). Considering that TBBPA-DHEE and TBBPA-MHEE potentially exist with very low concentrations in real environments, a sensitive impedimetric immunosensor can be developed for the detection of both trace organic containments on the basis of the biocatalysed precipitation strategy.

In this work, we reported an ultrasensitive competitive impedimetric immunosensor simultaneously detecting TBBPA-DHEE and TBBPA-MHEE, and the mechanism and assay protocol of our immunoassay is illustrated in Scheme 1. The competitive system was made up of the analyte, coating antigen (coated on glassy carbon electrode (GCE)/chitosan (CS)/MWCNTs@GONRs/ gold nanoparticles (AuNPs), modified electrodes) and primary antibody (Ab_1). Meanwhile, the amplification strategy was achieved by biocatalytic precipitation of 4-chloro-1-naphthol (CN) using horseradish peroxidase (HRP). To increase the sensitivity of the immunosensor, silica nanoparticles carrying SiO_2@PAA were introduced into the assay to increase HRP-loading (Huang et al., 2016; Qu et al., 2014; Zhao et al., 2016), accordingly, improving its catalytic performance. After the optimization of various parameters, our immunosensor was applied for the determination of the pollutants in an aquatic system.

2. Experimental section

Reagent/apparatus, synthesis of MWCNTs/GONRs, preparation of SiO_2@PAA@HRP, SiO_2@PAA@HRP@secondary antibody (Ab_2), the modified GCE, construction of the competitive-type immunoassay and sample collection were provided from S1–7.

3. Results and discussion

3.1. Characterization of AuNPs, MWCNTs, MWCNTs@GONRs and SiO_2@PAA, SiO_2@PAA@HRP and SiO_2@PAA@HRP@Ab_2

Fig. S2 showed a TEM image of the AuNPs, which were spherical with a diameter of approximately 25 nm. Fig. S3A and B displayed the TEM images of the pristine MWCNTs and well-prepared MWCNTs@GONRs, respectively. The untreated MWCNTs were thin, long, and tubular, forming a smooth structure without any impurities on their walls. In contrast, the diameter of the MWCNTs/GONRs was increased remarkably, and the rough edges of the graphene structures were observed on both sides of the nanotubes, which contributed to binding more biomolecular species stably. The provided SiO_2@PAA was spherical in shape and fairly uniform with a size of 50 nm (Fig. S4). Furthermore, a core-shell structure was observed, indicating that the SiO_2 core was covered by PAA brushes, which were further modified with HRP using the CCEE method based on their abundant carboxyl groups. As displayed in Fig. S5, after centrifugation of the synthetized SiO_2@PAA@HRP solution, the precipitate was dark brown, implying the successful combination of SiO_2@PAA and HRP. Besides, similar characteristic colour changes were also reported by the literature and our previous study (Fig. S5) (Li et al., 2017; Zhao et al., 2016), indicating the conjugation of SiO_2@PAA@HRP@Ab_2 was fulfilled as expected.

3.2. Impedimetric characteristics of the immunosensor

Electrochemical impedance spectroscopy (EIS) was used to study the interfacial characteristics of the electrodes during the modification process, Fig. 1 exhibited the Nyquist diagrams of variously modified electrodes in PBS buffer (pH 7) containing 5 mmol/L Fe $(CN)_6^{3-/4-}$ and 0.1 mol/L KCl. The complex impedance plots were presented in the form of a Nyquist plot (Zre vs. Zim), comprising of a linear part (accounting for a diffusion-limited process) and a semi-circular part (corresponding to an electron transfer-limited process, where the semi-circular diameter is equal to the electron transfer resistance, Ret.) (Hou et al., 2016; Peng et al., 2016). As seen in Fig. 1, after the bare GCE was firstly modified with CS, a natural cationic biopolymer with abundant amino groups that provided an efficient and stable electrode surface to bind MWCNTs@GONRs (Fang et al., 2014; Liang et al., 2012), a small semicircle domain (Fig. 1**b**) was observed in compassion with that of the bare GCE (Fig. 1**a**), which was ascribed to the generation of positive charges on the electrode surface (Janegitz et al., 2017). In addition, a clear decline in the charge transfer resistance value was found when the electrodes were functionalized with MWCNTs@GONRs (Fig. 1**c**) and AuNPs (Fig. 1**d**), suggesting that the modified electrodes possessed superior electrical conductivity. Then, after the respective electrode modifications with the antigen (Fig. 1**e**), antibody (Fig. 1**f**) and SiO_2@PAA@HRP@Ab_2 (Fig. 1**g**), an increase in the semicircle diameter was observed, corresponding to an increase in the interfacial charge transfer resistance (Ret), which was because the layers blocked the path of electron transport. Meanwhile, the modified electrodes increased the distance between the Fe $(CN)_6^{3-/4-}$ redox pair and the electrode surface, further leading to the observed increase in charge transfer impedance (Liang et al., 2017). Our results indirectly indicated successful modifications of the GCE, which was well prepared for the next step.

3.3. Verification of the amplification strategy

Various investigations were performed to evaluate the feasibility of the two-step amplification strategy of the immunosensor. In this study, according to our design, a signal enhancement was initially achieved by the HRP-biocatalysed precipitation of CN in the presence of hydrogen peroxide (H_2O_2), produced the insoluble benzo-4-chlorohexadienone on the modified electrode and hindered the electron transfer, resulting