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An approach for the preparation of highly sensitive electrochemical impedimetric immunosensors for the detection of illicit drugs

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ABSTRACT

In this paper, immunosensors based on a self-assembly modified (SAM) Au electrode for the specific detection of two illicit drugs, morphine (MO) and methamphetamine (MA), are reported. Using 3-mercaptopropionic acid SAM and activation by 1-ethyl-3-(3-dimethylaminoprophyl) carbodiimide (EDC)/n-hydroxysulfsuccinimide (NHS) with an adequate quantity of the antibody of MO or MA immobilized onto the surface to act as the sensing host, the sensors specifically respond to their respective target based on specific covalent bonding. Electrochemical impedance spectroscopy (EIS) is used as the read-out signal. The value of the electron transfer resistance (R_{et}) responded to the concentration of drug in spiked blood samples with an ultra-low detection limit and satisfactory recovery. In conclusion, the developed sensors are demonstrated to be more advantageous compared to those previously reported in terms of sensitivity.

1. Introduction

There is increasing interest in methods to detect low levels of chemicals because of their functionality, environmental effects and/or civil influence. Biosensors are a widely used, rapid and convenient analytic approach that play an important role in medical and biological fields, including forensic medicine [1,2]. Immunoassays are acknowledged to be a highly selective analytical method for several antigenic matters, including chemicals and biochemicals. The typical technique of immunoassays, such as ELISA, is widely applied, but the procedure is usually complicated, time-consuming, tedious and expensive [3], which limits the application of immunoassays for fast or in situ determinations [4]. As a more convenient device that holds the merits of biosensors and immunoassays, immunosensors have been reported in several fields, such as environmental science, food quality control and clinical diagnostics, in the past several decades [5]. This new technique, especially electrochemical immunosensors, has demonstrated great promise because of the simple instrumentation, high sensitivity, fast response, miniaturization, low cost and possible point-of-care testing [6,7].

The signaling origination of electrochemical immunosensor is rooted in the specific affinity between antibody and antigen [8], the capture of target antigen by an immobilized antibody on the electrode surface [9]. The self-assembled monolayer (SAM) offers one of the simplest methods for immobilization of an antibody among currently used methods, such as by silanized layer [10], polymer membrane [11], Langmuir–Blodgett film [12], Protein A [13] and nano-materials[14]. This method provides a reproducible, ultrathin and well-ordered layer that is suitable for further immobilization of antibodies [15].

A large number of electrochemical immunosensors have been developed, and various strategies were introduced into this field for read-out signals, such as potentials, currents or capacities [16]. Electrochemical impedance spectroscopy (EIS) has been used as a valuable tool for investigating the important parameters of electrode kinetics, conducting polymers, and semiconductors [17]. This method also provides interfacial information of the surface structure, such as chemo- or biosensors, because it provides details about the permeability or electron transference resistance [18]. With efficient power to character and signal of the interfacial transference of matter or electrons, EIS builds an excellent sensing platform for chemo/biosensors with high sensitivity, accuracy, and simplicity. Thus, the EIS technique has recently received particular attention [19,18]. Additionally, due to its dominance in regard to low interference and low cost, ease of miniaturization and label-

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free operation, especially for large molecules, EIS has recently become a promising candidate for applications in the area of immunosensors [20,21].

Morphine (MO), commonly presented as its hydrochloride, is a precursor of heroin. MO is the extract from unripe seeds or capsules of Papaver somniferum (Opium Poppy). In the medical field, MO is one of the narcotic drugs belonging to benzylisoquinoline alkaloids. MO can be rationally used as an effective sedative for medical purposes [22], but excessive use causes health problems, addictions, and even severe crimes and social problems [23]. Methamphetamine (MA, Ice), a typical psychostimulant drug that has been abused worldwide for decades, resulting in serious social problems. MA is now one of the most consumed illicit drugs of abusers. The toxicity of MA for the mammalian brain has been consistently reported to induce neurotoxicity, especially long-lasting changes in the central dopaminergic pathway. Therefore, a sensitive detection method is needed for the purposes of medical administration, industrial control, hard drug seizing and forensic judgment. Various methods have been developed to detect MO, such as LC with UV detection [24], TLC-MS [25], GC–MS [26], CE [27], HPLC [28], UPLC [29], and chemiluminescence [30]. The methods for MA detection include LLLME-CE [31], SPE-GCMS [32], EME-HPLC-UV [33], and SDME-DESI-MS [34]. These methods are commonly suffered from expensive instrumentation and time-consuming sample pre-treatment. Therefore, for the requirement of drug seizing and enforcement, a rapid, reliable, sensitive, even portable detection device for the detection of MO or MA is particularly important [35].

In this study, MO and MA immunosensors are developed. These sensors are constructed by the covalent bonding of a condensed corresponding antibody onto a 3-mercaptopropionic acid (3-MPA) SAM Au electrode after activation by n-hydroxysuccinimide (NHS)/3-(3-dimethylaminopropyl) – 1-ethylcarbodiimide hydrochloride (EDC) [36]. The analytical performances of the developed sensors were demonstrated by the detection of low concentrated MO or MA in spiked human serum. The results indicate that the acquired ultra-high sensitivity ensured the direct detection of the samples without any pre-treatment other than sufficient dilution.

2. Experimental

2.1. Materials and apparatus

MO injections were purchased from Northeast Pharmaceutical Group Co. Ltd. (Shenyang, China). Morphine antibody was obtained from Fankel Co. Ltd. (Shanghai, China). MA was provided by the Bureau of Public Security of Suzhou. MA antibody was obtained from Tiyo Biotechnology Co., Ltd. (Shanghai, China) (0.1 mg/mL). 3-Mercaptopropionic acid (3-MPA, $\geqslant$ 99%) was purchased from Alfa Aesar (Tianjin) Chemicals Co. Ltd. (Beijing, China). 3-(3-dimethylamino-opyl)-1-ethylcarbodiimide (EDC, 98%) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). N-hydroxysuccinimide (NHS, 98%) was purchased from Fluka (Buchs, Switzerland). Phosphate-buffered saline, 0.01 mol/L, (PBS, pH 7.4) was used to dilute all of the solutions. NaOH and KCl were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All reagents were used without further purification, and ultrapure water was used throughout the experiment.

The electrochemical measurements of cyclic voltammetry and electrochemical impedance spectroscopy were performed on an RST5200 Electrochemical Workstation (Suzhou Risetest Instrument Co. Ltd., Suzhou, China) with a three-electrode cell, including a saturated calomel electrode as the reference electrode, a Pt electrode as the counter electrode and an Au electrode (0.1 cm^2 area) as the basal working electrode. A warm box (Jinghong Instrument Co. Shanghai, China), was used to provide a required thermo-environment.

2.2. Preparation of the immunosensors

The gold electrode was polished to mirror-like with 1.0 μm alumina followed by 0.3 and 0.05 μm alumina slurry on a microcloth pad and subsequently cleaned with HNO_3, C_2H_5OH + NaOH and ultrapure water consecutively and dried by an N_2 stream. This pretreated Au electrode was later immersed in PBS solution containing 20 mmol L^{-1} 3-MPA for 12 h to perform the self-assembling modification. After removing excess 3-MPA by water flushing, the modified electrode was soaked in PBS buffer containing NHS and EDC for 4 h. After thoroughly rinsing with ultrapure water and drying by an N_2 stream, to obtain the full functionality, an excess amount of MO antibody or MA antibody solution (up to 200 μL of a 1:160 diluted solution with PBS, batch-wise) was dripped onto the surface of the modified electrode to ensure the covalent bonding of the antibody onto the modified layer in a wet environment at 30 °C for 40 min. The unbound antibodies were subsequently washed off with water. Finally, the sensor was dried by an N_2 stream and stored in a refrigerator at 4 °C.

2.3. The electrochemical measurement

A cyclic voltammetric scan within the potential range from −0.2 to 0.6 V with a scan rate of 50 mV/s in 0.01 mol/L KCl solution containing 1×10^{-3} mol/L $Fe(CN)_6^{4-/3-}$ electrochemical active probe, was applied to characterize the alteration of the electrode surface after each step of modification. Meanwhile, the EIS investigation (in the same solution, within the frequency range from 1 Hz to 100 kHz) output detailed information about the surface change in the modification process. As the most popular format of electrochemical impedance output, the Nyquist plot presents the imaginary component (capacitive reactance, Z'') against the real component (ohmic resistance, Z') at each excitation frequency. By its data-fitting with the equivalent circuit, from the diameter of the semicircle, the R_{et} (electron transfer resistance) can be read out as a quantitative signal for sensing the target because the antigen–antibody affinitive interaction disturbed the diffusion of the probe ions.

2.4. Regeneration and stability of the immunosensors

Immunosensor are commonly regenerated by removing the antigen from the antibody by disrupting their bonding with high ionic strength or highly acidic or alkaline solution. In this research, the resulting immunosensor was soaked in an NaOH–phosphate (pH 13.0) solution for 5 min to recover its sensing ability. In addition, the immunosensors were stored at 4 °C for several days and tested once a day to evaluate their stability by comparing the data with their original value.

2.5. The detection of target drug in spiked serum

The content of drugs in body fluid is a crucial index to distinguish illegal drug-taking. Therefore, the practicability of the immunosensors was evaluated by detecting MO or MA in spiked serum samples. Serum is the major component of the blood after removal of the blood cells (white and red blood cells) and clotting factor (blood platelets), namely, the blood plasma without the fibrinogens. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones and exogenous substances (e.g., drugs and microorganisms). Therefore, the dense blood components are the highest risk factors for interference with the determination. With our developed immunosensors, due to the ultra-high sensitivity without any means of pretreatment other than diluting the serum samples, the detection can be performed without interference from the