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A highly selective photoelectrochemical biosensor for uric acid based on core–shell Fe_3O_4@C nanoparticle and molecularly imprinted TiO_2

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ABSTRACT

Combining the surface modification and molecular imprinting technique, a novel photoelectrochemical sensing platform with excellent photochemical catalysis and molecular recognition capabilities was established for the detection of uric acid based on the magnetic immobilization of Fe_3O_4@C nanoparticles onto magnetic glassy carbon electrode (MGCE) and modification of molecularly imprinted TiO_2 film on Fe_3O_4@C. The developed biosensor was highly sensitive to uric acid in solutions, with a linear range from 0.3 to 34 μM and a limit of detection of 0.02 μM. Furthermore, the biosensor exhibited outstanding selectivity while used in coexisting systems containing various interferents with high concentration. The practical application of the biosensor was also realized for the selective detection of uric acid in spiked samples. The study made a successful attempt in the development of highly selective and sensitive photoelectrochemical biosensor for urine monitoring.

1. Introduction

Uric acid (UA), a primary final product of purine metabolism, is a key biomarker for the diagnosis of several diseases such as gout, Lesch–Nyhan syndrome, hyperuricemia, leukemia and so on (Li et al., 2012). Therefore, the monitoring of UA in human blood and urine is very important for the prevention of the mentioned and other similar diseases. Various techniques have been developed for UA detection, such as enzymatic assay (Piermarini et al., 2013), high-performance liquid chromatography (HPLC) (Kaňďár et al., 2011), mass spectrometry (Siekmann, 1985), capillary electrophoresis (CE) (Pormsila et al., 2009), chemiluminescence (Chaudhari et al., 2012), colorimetry (Miller and Oberholzer, 1990) and electrochemistry (Zhang and Yin, 2014; Guan et al., 2014). Despite many advances in UA detection, the sensitive, selective, stable and facile methods still need to be explored until now.

Photoelectrochemical measurement based on the electron transfer among analyte, photoactive species and electrode with photoirradiation is a newly and promising analytical technique for sensing application (Tu et al., 2010; Wang et al., 2009). In photoelectrochemical sensing, light is used to excite active species on the electrode, and the interaction between analytes and the illuminated photoelectrochemically active materials induces photocurrent change of the photoelectrochemical active species modified electrode. Coupling photoirradiation with electrochemical detection, the method exhibits the properties of both optical methods and electrochemical sensors (Zhang et al., 2013). Due to its separation of the excitation source and detection signal, the photoelectrochemical analysis possesses several advantages such as high sensitivity, low background signal, low cost and easy miniaturization of detection devices (Wang et al., 2014a, 2014b). The photoelectrochemical sensors have drawn growing interest in analytical chemistry, and have been utilized for sensitive analysis of target molecules and ions (Sun et al., 2013; Zhang et al., 2014; Jeon et al., 2013). Still, the photocatalytic oxidation lacks selectivity due to the nature of hydroxyl radicals or holes generated in photocatalytic oxidation, and is not suitable for selective analysis (Shi et al., 2011). Therefore, how to realize the selectivity of a photoelectrochemical sensor is a quite critical issue. Molecular imprinting technique, the design and construction of mimetic receptor system with predetermined recognition for target molecule, has been proposed and developed rapidly (Yang and Zhang, 2011, 2013). Recently, the photoelectrochemical sensors based on molecularly imprinted polymer have been fabricated, and exhibited a favorable selectivity towards template molecules (Sun et al., 2014; Tran et al., 2014; Wang et al., 2013).

In this work, Fe_3O_4@C nanoparticles and molecularly imprinted TiO_2 (MIT) were employed as carrier and sensing film, respectively. A novel photoelectrochemical biosensor with excellent photochemical catalysis and molecular recognition capabilities was fabricated for UA detection based on the magnetic immobilization of Fe_3O_4@C nanoparticles onto the MGCE and modification of MIT

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film on Fe_3O_4@C. The core–shell Fe_3O_4@C nanoparticles possess good magnetism and conductivity, which can facilitate the strong binding of carrier to the electrode and enhance the photocurrent (Zhang and Kong, 2011; Liu et al., 2014) due to its fast electron-transfer capability. The MIT is not only an outstanding photochemical catalyst with high surface area, structural rigidity, excellent stability and biocompatibility, but also can selectivity capture the target molecules (Yang et al., 2011; Luo et al., 2013). All these characteristics would lead to an excellent photoelectrochemical biosensor with high sensitivity, selectivity and stability.

2. Experimental

2.1. Materials and apparatus

$FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, NH_4OH solution (25%) and benzoquinone (BQ) were of analytical grade and obtained from Shanghai Chemical Reagent Co. Ti(O-^{n}Bu)$_4$ was purchased from Sigma Chem. Co. and used as received. Glucose, UA, ascorbic acid (AA), glutamic acid (GA), cytosine, purine and urea were purchased from Shanghai Aladdin Chemical Co., China. All other chemicals were of analytical grade and used without further purification. Deionized (DI) water (resistivity of 18 MΩ cm) was obtained from a Millipore Milli-Q Water System (Millipore Inc.), and was used for rinsing and for makeup of all aqueous solutions.

Transmission electron microscopy (TEM) analysis was performed on a JEOL 2100F electron microscope (Japan) at an accelerating voltage of 200 kV. Scanning electron micrograph (SEM) was conducted on a Hitachi S-520 scanning electron microscope (Hitachi Ltd., Tokyo, Japan) working at 20 kV. X-ray diffraction (XRD) was performed on a Rigaku D/MAX-RCX-ray diffractometer using Cu Kα radiation. Infrared (IR) spectra were recorded on Nicolet 200SXV Fourier transform infrared (FTIR) spectrometer using a KBr wafer. The magnetization measurements were performed at room temperature using model 155 vibrating magnetometer. The chromatographic analysis was performed in High Performance Liquid Chromatography (HPLC) system equipped with a variable-wavelength ultraviolet (UV) detector and an automatic injector (LC-10 VP, Shimadzu Scientific Instrument, Kyoto, Japan). A 320 W low-pressure mercury lamp (Philips, 254 nm) was used as UV irradiation source. All electrochemical experiments were carried out on CHI660c workstation (CH Instrument, USA).

2.2. Design and fabrication of the UA photoelectrochemical biosensor

The preparation of Fe_3O_4@C nanoparticles were conducted according to the previous reports with some modification (Zhang and Kong, 2011). Briefly, 25% NH_4OH solution (10 mL) was slowly dropped into a 30 mL aqueous solution containing 1.85 mmol $FeCl_2 \cdot 4H_2O$ and 3.7 mmol $FeCl_3 \cdot 6H_2O$ in N_2 atmosphere under vigorous stirring. After incubation for 30 min at 80 °C, the resultant ultrafine magnetic particles were collected by magnetic decantation, washed several times with DI water, and then vacuum dried at 60 °C for 4 h. The as-prepared Fe_3O_4 nanoparticles (50 mg) were dispersed in a 0.1 M HNO_3 solution for 10 min, followed by washing several times in DI water. Subsequently, the treated magnetic nanoparticles were introduced into 0.3 M aqueous glucose solution (15 mL). After vigorous stirring for 20 min, the solution was sealed in a Teflon-line autoclave. The autoclave was kept at 180 °C for 6 h, and then cooled naturally. The resultant products were separated by a magnet and washed in DI water and ethanol. Finally, dried at 60 °C, stand by to application.

The MGCE was prepared and treated according to our previous work (Yang et al., 2014). 2.0 mL aqueous suspension of Fe_3O_4@C (0.3 mg mL^{-1}) was dripped onto the obtained MGCE (10 mm in diameter), the solid Fe_3O_4@C nanoparticles were magnetically attracted down to the electrode surface immediately from the suspension. Thus, Fe_3O_4@C modified MGCE was fabricated.

The formation of MIT film on Fe_3O_4@C modified MGCE was performed by sol–gel hydrolysis of Ti(O-^{n}Bu)$_4$ followed by extraction and heat treatment. Specifically, Ti(O-^{n}Bu)$_4$ (0.8 mmol) and UA (0.3 mmol) were dissolved in 10 mL of toluene–ethanol (v/v, 2:1) mixture, and then vigorously stirred at room temperature. Subsequently, the solution was diluted by 20 times with water-saturated toluene and continuously stirred for 6 h to get a sol–gel solution which was used as a dipping solution. The Fe_3O_4@C modified MGCE was immersed into the dipping solution for 8 min at room temperature, rinsed with toluene, hydrolyzed in water and dried in N_2 atmosphere. The "dipping-rinse-hydrolyzation-drying"cycle was repeated two times, and then the Fe_3O_4@C modified MGCE whose surface was covered with a TiO_2 layer containing the template molecule of UA was obtained. Finally, the template UA molecules were completely removed from the modified layer by multiple extractions ($n=8$, extraction time 10 min) with 10 mL hot water (ca. 80 °C). After heat treatment at 200 °C in N_2 atmosphere for 4 h, the MIT modified electrode was obtained and denoted as Fe_3O_4@C@MIT modified MGCE. Thus, the UA photoelectrochemical biosensor was fabricated. The obtained biosensor was stored dry at room temperature for future use. For comparison, the formation of non-imprinted TiO_2 on Fe_3O_4@C modified MGCE was conducted using a similar procedure without template molecules, and was denoted as Fe_3O_4@C@TiO_2 modified MGCE.

2.3. Photoelectrochemical measurements

All photoelectrochemical measurements were performed in a three-electrode cell configuration with Fe_3O_4@C@MIT or Fe_3O_4@C@TiO_2 modified MGCE as the working electrode, a saturated calomel electrode as the reference electrode, and a Pt wire counter electrode. After the electrodes were incubated in 0.1 M phosphate buffer solution (PBS, pH 7.4) containing different concentration of UA for 10 min under stirring, the UV illumination was conducted and the photocurrent was generated due to photocatalytical oxidization of UA. *I–t* curve was used for sensitivity, selectivity, stability and detection experiments.

3. Results and discussion

3.1. Fabrication and characterization of the UA photoelectrochemical biosensor

The route of design and fabrication of the UA photoelectrochemical biosensor was shown in Fig. 1 A. Fe_3O_4 nanoparticles were prepared by co-precipitation of Fe^{2+} and Fe^{3+} ions in ammonia solution. The carbon layer was formed at the surface of Fe_3O_4 nanoparticles by the hydrothermal reaction in aqueous glucose solution. The as-formed core–shell Fe_3O_4@C nanoparticles were modified onto the MGCE surface by magnetic force. The molecularly imprinted TiO_2 was functionalized on the Fe_3O_4@C modified MGCE by the sol–gel hydrolysis of Ti(O-^{n}Bu)$_4$ in the presence of the template molecules UA. UA was embedded in TiO_2 film by strong hydrogen bond formed between N–H groups of UA and hydroxyl groups of TiO_2. After removal of UA, recognition sites complementary to the molecular shape, size and functionality of UA were formed in TiO_2 film which would efficiently and selectively rebind UA in solution. The morphology of the as-prepared samples was investigated by TEM (Supplementary information Fig. S1). As presented in Fig. S1a, the Fe_3O_4@C microspheres showed a