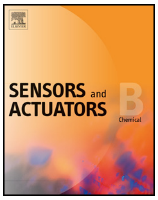
Sensors and Actuators B 230 (2016) 449–455

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb



Nonenzymatic electrochemiluminescence glucose sensor based on quenching effect on luminol using attapulgite–TiO_2

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ARTICLE INFO

Article history:
Received 13 September 2015
Received in revised form 1 February 2016
Accepted 6 February 2016
Available online 10 February 2016

Keywords:
Nonenzymatic
Electrochemiluminescence
Glucose
Att–TiO_2
Luminol

ABSTRACT

A new nonenzymatic glucose ECL sensor based on attapulgite (Att) integrated with semiconductor material TiO_2 was developed. The prepared material was characterized by transmission electron microscopy (TEM), X-ray diffractometer (XRD), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Based on ECL experimental results, Att–TiO_2 composites were found to be able to improve the ECL properties of luminol. When glucose is added into the system, TiO_2 NPs serve as a catalyst and dissolved O_2 acts as a cosubstrate for the glucose oxidation reaction. Dissolved O_2 also worked as coreactant for the luminol ECL emission. Due to the consumption of dissolved O_2, a quenching effect would appear on the luminol ECL emission. Under the optimized conditions, the linear logarithmic relationship between ECL intensity and the concentration of glucose was valid in the range from 1.0 mM to 1.0 nM ($R = 0.9976$) with a detection limit (S/N = 3) of 10.0 pM. In addition, the proposed sensor presented good reproducibility, stability, and sensitivity for glucose detection and can be successfully applied in the determination of glucose in real blood samples.

1. Introduction

Glucose is the primary energy source of the body. The level of glucose in blood has been used for diagnosis of diabetes or hypoglycemia. Besides the need in glucose monitoring in the case of diabetes patients, it is also essential for non-diabetic acute care patients in order to control glucose levels [1]. Therefore, the development of fast, sensitive, selective and reliable methods for glucose monitoring is important in clinical diagnostics, food industry and biotechnology [2]. Electrochemiluminescence (ECL), a special form of chemiluminescence (CL) in which light emission is generated by electrochemical reactions, has been receiving higher and higher attention because of its excellent sensitivity, low background signal and some additional advantages [3], since Haghighi and Bozorgzadeh reported ECL behaviors of Si for the first time in 2002 [4]. Among various ECL systems, luminol is considered as one of the most popular ECL luminophor due to its low oxidation potential, inexpensive reagent consumption and the high emission yields [5,6]. So, special attention had focused on the ECL studies concerning luminol for glucose analysis [7]. Luminol-based ECL glucose biosensors on the oxidation of glucose catalyzed by glucose oxidase have the advantages of being a simplified and sensitive instrument. However, these so-called biosensors have poor stability because glucose oxidase (GOx) quickly loses its activity at below pH 2 and above pH 8 and when temperature is above 40 °C. In addition, these compounds suffer from badly damage or time consuming due to their long-winded fabrication procedure for the fixing enzyme onto the electrode [8]. Therefore, researchers have focused on nonenzymatic glucose ECL sensors. Currently, only four literatures have reported the corresponding nonenzymatic ECL behavior of luminol to determine glucose [9–12]. It is desirable to discover more materials to develop effective enzyme-less luminol-based ECL biosensors of detecting glucose.

As far as we know, the ECL intensity is greatly influenced by the property of the working electrode, especially the material's surface property. Titania dioxide (TiO_2), an important semiconductor material with a large surface area, has previously been researched for photo-assisted degradation of a variety of toxic chemicals.

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http://dx.doi.org/10.1016/j.snb.2016.02.026
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Titania is known to be an effective catalyst for the cleavage of diols when reactive oxygen species (ROS) is used as oxidant [13]. Therefore, it would be a good working electrode for ECL detection and has attracted increasing interest in recent years [14]. Because the modified TiO_2 nanocrystals are easy to fall off from the electrode surfaces, the electrodes have poor stability and the ECL intensity is not strong enough [15]. Considering the utilization and conversion of abundant negatively charged product, attapulgite (Att), which helps to form relatively high surface area and moderate cation exchange capacity [16] due to its permanent negative charges on its surface, has been successfully used to make TiO_2 more active. There are large reserves of Att in Huai'an, China. The specific property and large distribution of Att make it to be studied undoubtedly.

In present paper, we combined the huge specific surface area of Att, which can fix TiO_2 onto the electrode surface more stable and prevent the deposition of luminol oxidation products. In addition, we found that AttTiO_2 nanohybrids could effectively enhance luminol's ECL in experimental process. The obtained sensor exhibited very sensitive ECL quenching responses for detection of glucose. The analytical procedure of this ECL sensing is illustrated in Scheme 1. First, Att–TiO_2 composites material was prepared, then anchor it onto the surface of GCE, this composite can improve the ECL properties. While the glucose was added into the solution, glucose was oxidized in the presence of TiO_2, and part of oxygen radicals ($O_2{}^{\bullet}-$) were consumed, so that the ECL signal was quenched. The intensity of quenching signal was linearly associated with the logarithm of the concentration of glucose, based on it, the detection of glucose could be achieve. The stable and strong ECL emission guarantees that Att–TiO_2 composite works as a sensitive sensor.

2. Experimental

2.1. Reagents and chemicals

Luminol was obtained from J&K Chemical. Glucose and KH_2PO_4 were received from Sinopharm Chemical Reagent Co., LTD. (Shanghai, China). $K_3[Fe(CN)_6]$ was purchased from Guangdong Shantou West Long Chemical Plant. $K_4[Fe(CN)_6]\cdot 3H_2O$ was obtained from Wuxi City and Yasheng Chemical. NaOH was from Nanjing Chemical Reagent Co., LTD. Phosphated-buffered solution (PBS) (pH 7.4, 0.1 M) was prepared using 0.1 M KH_2PO_4 and 0.2 M NaOH. A 1.0 mM luminol (3-aminophthalhydrazide) stock solution was prepared by dissolving it in a small amount of 0.1 M NaOH. A stock solution of glucose (1.0 mM) was prepared in PBS and stored at 4 °C when it was not in use. The stock solution of glucose was allowed to mutarotate at room temperature for 24 h before use. Different concentrations of working solutions were diluted step by step with PBS. Human serum samples were kindly provided by Huai'an First People's Hospital. All chemicals were of analytical reagent grade and were used without further purification. All solutions were prepared exclusively in double distilled water.

2.2. Synthesis of Att–TiO_2 nanohybrids

The nanomaterials of Att–TiO_2 were prepared by a similar route to the technique reported before [17]. The first step is the simple purification of Att. 1.0 g of raw Att when it was dispersed in 100 mL distilled water and ultrasound for 0.5 h, then the nether sand and large stones were removed. This produced a homogeneous Att suspension which was used directly in the later modification section without further acid activation. Secondly, 3.4 g of $Ti(OBu)_4$ was added to the as-prepared Att suspension, and then 100 mL distilled water was added drop by drop to let the $Ti(OBu)_4$ hydrolyze in situ to deposit $Ti(OH)_4$ onto the surface of Att. After vigorous stirring for 4 h, the suspension was filtered, washed with water, dried at 80 °C and calcined at 300 °C for 4 h. After grinding to about 200 meshes in a carnelian mortar, TiO_2 oxides coated onto Att were obtained and labeled as Att–TiO_2.

2.3. Instrumentation and procedures

The morphologies of the Att–TiO_2 nanohybrids were characterized by transmission electron microscopy (TEM) (JEM-3011, JEOL, Japan). The crystal structures of the samples were examined by an X-ray diffractometer (XRD, BrukerD8, Advanee, Germany) with Cu Kα radiation (λ) 0.15406 nm. Electrochemical impedance spectroscopy (EIS) measurements were monitored with an Autolab PGSTAT 30 Analyzer (Metrohm Autolab B.V., Switzerland) in a solution containing 5 mM $Fe(CN)_6{}^{3-/4-}$ and 0.1 M KCl. The frequency was ranged from 0.1 to 100,000 Hz with an alternating current voltage of 10 mV. Cyclic voltammetry (CV) and ECL measurements were taken using a homemade ECL/EC system. Cyclic voltammetrys (CVs) were carried out using an electrochemical working station (CHI 660D, Chenhua Inc., China) in 0.1 M KCl solution containing 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ and in PBS (pH 7.4) for the bare GCE and Att–TiO_2-midified GCE. The ECL signals were acquired by a MPI-M multifunctional analytical system (Xi'an Remax Electronic Science Tech. Co., Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT), which is used for transforming ECL emission into electrical signals, set at 600 V in the process of detection and the ECL detector cell was placed in front of the PMT. The detector cell was made of a microbeaker (high: 35 mm, i.d.: 25 mm) and we performed the ECL measurements in 3 mL PBS (pH 7.4) with 0.35 mM luminol and 1.0 μM glucose. In addition, all electrochemical experiments were performed with a conventional three-electrode set-up where glassy carbon electrode was used as working electrode, platinum wire as counter electrode and Ag/AgCl (saturated KCl solution) as reference electrode, respectively. All measurements were performed at room temperature.

2.4. Electrodes preparation and modification

A glassy carbon electrode (GCE, 3 mm diameter) was used for the preparation of the nonenzymatic glucose ECL biosensor. Before electrode modification, the GCE was polished with 0.3 and 0.05 μm alumina paste (Gao Shi Rui Group Technology Co., LTD., Wuhan, China) on chamois leather and carefully rinsed with 1:20 (v/v) nitric acid, ethanol and doubly distilled water in an ultrasonic bath to give a smooth and clean electrode surface. Next, 1.0 mg of Att–TiO_2 was dispersed in 1.0 mL double distilled water with ultrasonic agitation for 1 h to achieve a well-dispersed suspension. Then, a 5.0 μL of the prepared Att–TiO_2 suspension was pipetted on the surface of GCE to construct Att–TiO_2-modified GCE and allowed to be dried in air at room temperature.

3. Results and discussion

3.1. Characterization of Att–TiO_2 nanocomposites

Fig. 1A shows the XRD patterns of Att (a), TiO_2 (b) and Att–TiO_2 (c). The reflections of Att (a) at $2\theta = 8.34^\circ$, 19.8°, 26.6°, 28.0° and 34.9° were consistent with the standard spectrogram Att (JCPDS No. 37-0783) structure. Their corresponding crystal face index are (1 1 0), (0 4 0), (2 3 1), (4 0 0) and (1 0 2) respectively. The reflections (b) at $2\theta = 25.3^\circ$, 37.8° and 48.0° corresponded to TiO_2 that is consistent with the standard spectrogram TiO_2 (anatase) (JCPDS No. 65-5714) structure. Their corresponding crystal face index are (1 0 1), (0 0 4) and (2 0 0). Montmorillonite and quartz were also found. The characteristic reflections of Att were observed in the