Sensors and Actuators B 213 (2015) 5–11

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

An ultrasensitive electrochemiluminescence sensor for detecting diphenhydramine hydrochloride based on L-cysteine-functionalized multiwalled carbon nanotubes/gold nanoparticles nanocomposites

Chongchong Miao, Amin Zhang, Yanan Xu, Shen Chen, Feimeng Ma, Chusen Huang, Nengqin Jia*

The Education Ministry Key Laboratory of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials and Shanghai Municipal Education Committee Key Laboratory of Molecular Imaging Probes and Sensors, Department of Chemistry, Shanghai Normal University, Shanghai 200234, PR China

ARTICLE INFO

Article history:
Received 31 December 2014
Received in revised form 6 February 2015
Accepted 13 February 2015
Available online 21 February 2015

Keywords:
Electrochemiluminescence sensor
Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate
MWCNTs-Cys-AuNPs
Diphenhydramine hydrochloride detection
Pharmaceutical analysis

ABSTRACT

A new electrochemiluminescence (ECL) sensor was constructed for the ultrasensitive detection of diphenhydramine hydrochloride (DPH) based on L-cysteine-functionalized multiwalled carbon nanotubes/gold nanoparticles (MWCNTs-Cys-AuNPs) nanocomposites. In this work, the nanocomposites were facilely synthesized by using L-cysteine as linking agent which could covalently conjugate to MWCNTs and AuNPs via amide bond and Au—S bond. Herein, the prepared MWCNTs-Cys-AuNPs were used as matrix material and fixed by Nafion to adsorb $Ru(bpy)_3^{2+}$ on glassy carbon electrode (GCE) to provide a new ECL sensor for DPH. Then the electrochemical and ECL behaviors of the sensor were investigated. Approximate 9-fold enhancement in ECL intensity was observed after addition of DPH. By optimizing the experimental conditions, the proposed ECL sensor exhibited excellent assay performances in determination of DPH with a wide linear range (2×10^{-8}–7.5×10^{-4} M), low detection limit (6.7×10^{-9} M), good reproducibility and acceptable stability. Furthermore, the constructed ECL sensor was used to analyze the DPH in real sample with a satisfactory result, which holds a great potential in the field of pharmaceutical analysis.

1. Introduction

Electrochemiluminescence (ECL), also called electrogenerated chemiluminescence, is a process that the luminescence generated by converting electrochemical energy into radiative energy on the surface of electrode during the electrochemical reaction [1,2]. Recently, ECL has received considerable attention because of its versatility, good temporal, spatial control and simplified optical setups [3]. With the combination of inherent advantages of electrochemistry and chemiluminescence, ECL technology has been widely used in various research fields such as chemical sensing [4,5], imaging [6] and optical studies [7]. Among all the ECL luminophores (e.g. luminol, acridinium easter, metal nanoclusters, etc.), $Ru(bpy)_3^{2+}$-based ECL has obtained tremendous attention since it has reversible electrochemical behavior, high luminescent quantum efficiency, wide linear range and good solubility in a variety of aqueous and non-aqueous solvents [8]. Meanwhile, compared with the $Ru(bpy)_3^{2+}$ ECL system in solution phase, solid-state ECL showed several advantages such as reducing consumption of expensive reagent, simplifying experiment design and improving the separation efficiency of samples from the combined flow system [9,10]. Moreover, the immobilized $Ru(bpy)_3^{2+}$ on the surface of electrode could be recyclable during the ECL reaction.

Multiwalled carbon nanotubes (MWCNTs) were first discovered by Iijima in 1991 [11]. Since then, their extraordinary mechanical, electrical, thermal, chemical, and structural properties have attracted intensive attention [12–15]. P electrons on carbon atoms of MWCNTs can form delocalized π bond and remarkable conjugative effect, which makes MWCNTs display a good electrical property. And a variety of MWCNTs-based electrochemical sensors have been developed [16–18]. Gold nanoparticles (AuNPs) also have been widely used in biosensors due to its facile synthesis, good biocompatibility, large surface area, excellent conductivity and peculiar electro-optical properties [19–21].

Diphenhydramine hydrochloride (DPH), one kind of histamine H1-receptor antagonist, has been widely used as antiallergic,

* Corresponding author. Tel.: +86 21 64321045; fax: +86 21 64321833.
E-mail address: nqjia@shnu.edu.cn (N. Jia).

http://dx.doi.org/10.1016/j.snb.2015.02.069
0925-4005/

antiemetic and antitussive drugs. It has also been used for the control of Parkinsonian symptoms and treatment of nausea and vomiting [22]. But super doses of DPH can cause lethargy, heart palpitations, muscle tremors, blurred vision, confusion and even seizures and other toxic reactions. Generally, DPH is made into tablets, capsules and injections, in which the content of DPH should be accurately controlled during the process of drug preparations. Currently, there are several approaches available for monitoring DPH such as flow injection spectrophotometry (FISP) [23], capillary electrophoresis (CE) [24], spectrophotometric method (SPM) [25], high-performance liquid chromatography (HPLC) [26] and screen-printed electrode (SPE) [27]. Despite the improvement of these methods, it is still desirable to develop a simple, fast, sensitive and low-cost approach for the detection of DPH. What is more, there are little reports using ECL methods for monitoring DPH in drug preparations.

In this paper, a new designed $Ru(bpy)_3^{2+}$-based ECL sensor for the ultrasensitive detection of DPH was described. MWCNTs-Cys-AuNPs nanocomposites were facilely prepared by using L-cysteine as linking agent, in which L-cysteine can be covalently conjugated to the carboxylic group in MWCNTs via amide bond and AuNPs through Au—S bond. Due to the remarkable conductivity and large surface area of the synthesized nanocomposites, the proposed ECL sensor showed a wide linear range, low detection limit, good reproducibility and acceptable stability. Moreover, it had been successfully applied to the determination of DPH in real drug samples.

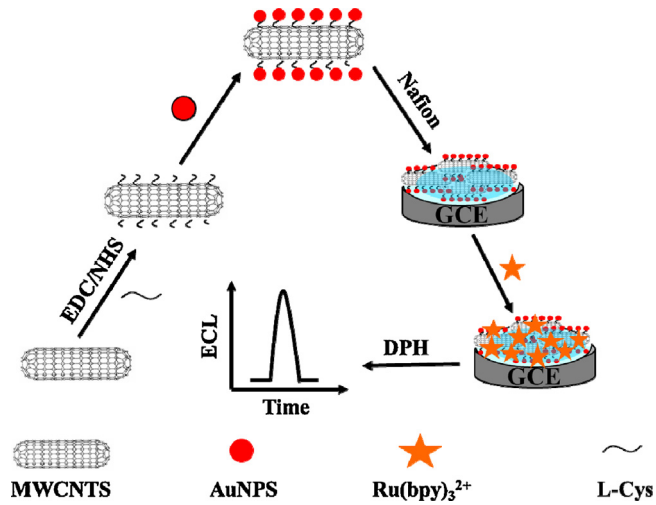
2. Experimental

2.1. Materials and reagents

Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), diphenhydramine hydrochloride (DPH) (≥98%), 1-ethyl-(3-dimethylaminopropyl) carbondiimide hydrochloride (EDC) and N-hydrooxysuccinimide (NHS), Nafion (5 wt%) were purchased from Sigma–Aldrich. Mutiwalled carbon nanotubes (MWCNTs) (>95% purity) were obtained from Shenzhen Nanotech Port Co. (China). L-Cysteine (L-Cys) was obtained from Aladdin. $HAuCl_4 \cdot 4H_2O$ (≥47.8%) and Trisodium citrate (≥98%) were obtained from Shanghai Chemical Reagent Co., Ltd. All other chemicals were of analytical grade and used without further purification. The phosphate buffer solutions (PBS) were prepared by mixing Na_2HPO_4 and NaH_2PO_4. Double-distilled water was used throughout the experiment.

2.2. Apparatus

The morphology and sizes of MWCNTs and MWCNTs-Cys-AuNPs were estimated from transmission electron microscopy (TEM) (Hitachi model H-800 TEM, opened at an accelerating voltage of 100 kV). Scanning electron microscopy (SEM) image was obtained using a LEO1530 field emission SEM system (Germany). ECL was recorded with a model MPI-E ECL analyzer (Xi'an Remax Electro-Science & Technology Co. Ltd., China) with the voltage of the photomultiplier tube (PMT) set at 600 V. CVs measurements were performed with a CHI660B electrochemistry work-station (Shanghai Chenhua Apparatus Inc., China). A conventional three-electrode setup contained a modified glassy-carbon electrode (GCE, 3.0 mm in diameter) as working electrode, a platinum auxiliary electrode, and an Ag/AgCl (3 M KCl) reference electrode. The pH measurements were made with a pH meter (FE 20, Mettler-Toledo, Switzerland).



Scheme 1. Schematic fabrication process of ECL sensor.

2.3. Preparation of AuNPs

AuNPs were prepared by a well-established citrate-reduction method [28]. Prior to experiment, all glassware used in the following procedures was cleaned in a bath of freshly prepared HNO_3/HCl (3:1) solution. The glassware should rinse thoroughly with distilled water and dried in air. Then a beakerflask that contained 100 mL $HAuCl_4 \cdot 4H_2O$ (0.01%) was heated to boiling under vigorous stirring. Sodium citrate solution (0.01 g/mL, 2 mL) was rapidly introduced into the beakerflask and reacted with $HAuCl_4$. The color of the solution changed from pale to dark blue and then deep purple and finally wine red, which indicated the formation of AuNPs. Subsequently, the solution was allowed to boiling for another 15 min to form steady colloid and then the heater was removed. The resulting solution was permitted to cool down at room temperature and stored at 4 °C until use.

2.4. L-Cysteine-mediated functionalization of MWCNTs and synthesis of MWCNTs-Cys-AuNPs nanocomposites

L-Cysteine was used to covalently conjugate to acid-treated MWCNTs through amide bond. In a typical procedure, a proper amount of MWCNTs were firstly treated with 3:1 (v/v) H_2SO_4/HNO_3. After sonicated for 16 h, the resulting dispersion was high-speed centrifuged and washed with water and ethanol repeatedly until the pH of centrifugate was about 7.0. The carboxylic group-functionalized and shorter MWCNTs were dried at 60 °C in a vacuum oven. Then 15 mg acid-treated MWCNTs were redispersed in 5 mL pure water, then EDC (20 mg), NHS (20 mg) and L-cysteine (40 mg) were added, sonicated to a well-distributed solution. Afterwards, the pH of the mixture was adjusted to 7–8 by 0.1 M NaOH. Finally, the mixture was allowed to react for 24 h with a shaker to obtain the Cysteine-functionalized MWCNTs (MWCNTs-Cys). The MWCNTs-Cys were separated by centrifugation and dried in air.

MWCNTs-Cys-AuNPs nanocomposites were synthesized as follows. 10 mg as-prepared MWCNTs-Cys were added into 50 mL AuNPs solution under shaking overnight. The MWCNTs-Cys-AuNPs nanocomposites were then obtained after centrifugation, washed with distilled water and dried at room temperature.

2.5. Construction of the ECL sensor

The construction process of the ECL sensor was shown in Scheme 1. Firstly, GCE was carefully polished with 0.3 and 0.05 μm