Materials Science and Engineering C 74 (2017) 27–35

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Fabrication of highly sensitive gold nanourchins based electrochemical sensor for nanomolar determination of primaquine

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ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 1 November 2016
Accepted 16 December 2016
Available online 7 January 2017

Keywords:
Gold nanourchins
Glassy carbon electrode
Voltammetry
Primaquine
Pharmaceutical formulations
Human urine

ABSTRACT

A gold nanourchins modified glassy carbon electrode (AuNu/GCE) was developed for the determination of antimalarial drug, primaquine (PQ). The surface of AuNu/GCE was characterized by electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cyclic voltammetry (CV). EIS results indicated that the electron transfer process at AuNu/GCE was faster as compared to the bare electrode. The SEM and TEM image confirmed the presence and uniform dispersion of gold nanourchins on the GCE surface. Upon investigating the electrochemical behavior of PQ at AuNu/GCE, the developed sensor was found to exhibit high electrocatalytic activity towards the oxidation of PQ. Under optimal experimental conditions, the sensor showed fast and sensitive current response to PQ over a linear concentration range of 0.01–1 μM and 0.001–1 μM with a detection limit of 3.5 nM and 0.9 nM using differential pulse voltammetry (DPV) and square wave voltammetry (SWV), respectively. The AuNu/GCE showed good selectivity, reproducibility and stability. Further, the developed sensor was successfully applied to determine the drug in human urine samples and pharmaceutical formulations demonstrating its analytical applicability in clinical analysis as well as quality control. The proposed method thus provides a promising alternative in routine sensing of PQ as well as promotes the application of gold nanourchins in electrochemical sensors.

1. Introduction

Primaquine (PQ; chemically known as N-(6-methoxyquinolin-8-yl)pentane-1,4-diamine; Fig. 1) is an 8-aminoquinoline commonly used as an antimalarial drug. First synthesized in 1940s by Robert Elderfield, PQ is the only FDA approved drug that can eliminate the intra-hepatic forms (schizonts and hypnozoites) of *P. vivax* and *P. ovale* [1]. It exhibits significant gametocytocidal activity in P. falciparum malaria substantially lowering the risk of transmission [2]. PQ is also suggested for causal and terminal prophylaxis in vivax malaria [3]. In addition, the drug is used for the treatment of mild to moderate cases of pneumocystis pneumonia in AIDS patients [4]. Though PQ is highly effective and largely recommended for management of falciparum and vivax malaria, there have been reports about potential haemolytic toxic effects associated with its use in patients with G6PD deficiency [5]. Other common side effects of the drug are gastrointestinal disorders, headache, cardiac arrhythmia, leucopenia, hypertension and methemoglobinemia [6–9]. Considering the importance of drug analysis in quality control and therapeutic drug monitoring, it is imperative to develop cheap, robust and accurate analytical methods to determine PQ in pharmaceutical samples and human body fluids.

Various methods have been developed for PQ determination, such as spectrophotometry, mass spectrometry, chromatography, electrochemical methods, colorimetry and fluorimetry [10–18]. Among these, the voltammetric methods have proved efficacious for detection of several organic and inorganic compounds on account of their selectivity, sensitivity and reliability at a low cost, with the instrumentation having potential for miniaturization and automation [19,20]. So far, only two voltammetric methods have been reported in literature for quantification of PQ exhibiting detection limits in micromolar range [15,21]. Hence, the need to develop a more sensitive, selective and accurate electroanalytical method for trace analysis of PQ is significant.

Last few decades have witnessed significant progress in the development of electrochemical sensors based on the use of a range of nanoparticles modified electrodes. Various metallic nanomaterials with enhanced electrical properties have been synthesized [22–30]. Gold nanoparticles (AuNPs) have revealed great potential application in the field of electroanalysis due to their unique properties, such as excellent conductivity, chemical stability, resistance to corrosion, high surface area and catalytic activity [31]. When immobilized onto an electrode surface, AuNPs increase the surface area of the modified electrode and improve the electron transfer rate providing sensitive and selective systems for the detection of various analytes. Since AuNPs are considered

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http://dx.doi.org/10.1016/j.msec.2016.12.126
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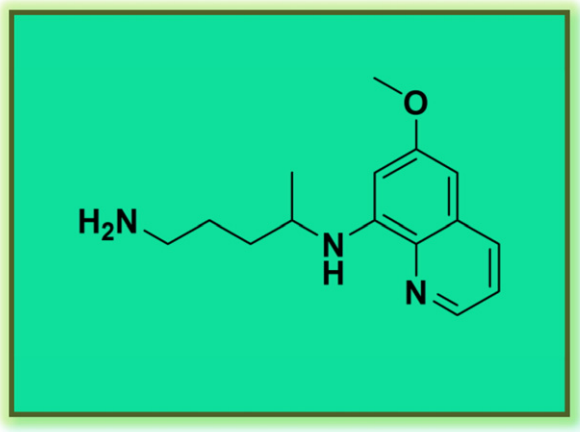

Fig. 1. Chemical structure of Primaquine.

as ideal candidates for constructing electrochemical sensors, these were chosen over other metallic particles for the proposed work. Among them, gold nanourchins that are endowed with a rough surface and urchin-like morphology are worth investigating. Normal AuNPs are spherical in shape. However, the gold nanourchins have urchin-like shape i.e., spherical shaped structure with spikes or needles coming out of the surface. This provides the nanourchins with more active surface area and hence the gold nanourchins modified electrode shows higher electrocatalytic activity as compared to simple gold nanoparticles modified electrode. Thus, using gold nanourchins as electrode modifier improves the sensitivity of the electrode to a greater extent and hence, a much lower concentration of the analyte can be detected. These nanostructures are suggested to have high surface to volume ratio thus making them an exceptional candidate for electrode modification [32,33]. The presence of spike shaped protrusions in nanourchins increases the effective surface area of the electrode resulting in improved electrochemical response as compared to spherical shaped gold nanoparticles modified electrode. The concept of employing urchin-shaped gold nanoparticles as an electrode modifier in voltammetry is very new. Literature survey reveals extremely few reports on the use of gold nanourchins based electrode for detection of an organic or inorganic compound. The present study describes the first-time use of a gold nanourchins modified glassy carbon electrode for voltammetric determination of PQ, an antimalarial drug. The novelty of the work thus lies in the use of gold nanourchins as an electrode modifier for determination of primaquine. The developed electrochemical sensor was found to exhibit significant electrocatalytic activity towards PQ oxidation and was successfully evaluated for determination of PQ in real samples.

2. Experimental

2.1. Materials

Primaquine bisphosphate and gold nanourchins were purchased from Aldrich. Other chemicals employed were of analytical grade and used as received (without further purification). Phosphate buffer solution (PBS) was prepared from 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 and employed as the supporting electrolyte. Stock solution of PQ (2.0 mM) was freshly prepared in water prior to measurements to avoid any decomposition. All aqueous solutions were prepared using double-distilled water.

2.2. Apparatus

The morphology of the modified electrode was observed using transmission electron microscopy (TEM) (JEOL 1010, USA) and scanning electron microscopy (SEM) (LEO 1450 Ultra Plus, Zeiss, Germany). The electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) experiments were carried out using a CHI660E electrochemical workstation (CH Instruments, USA). A conventional three electrode system was utilized throughout the experiments which comprised of a bare or modified GCE (3.0 mm in diameter) as the working electrode, a platinum counter electrode and an Ag/AgCl reference electrode. All the three electrodes were provided by CH Instruments, USA. The pH measurements were performed using EUTECH cyber scan pH 510 bench meter. All the electroanalytical measurements were performed at room temperature (25 ± 2 °C). Instrumental conditions for DPV were: scan rate 100 mVs^{-1}, pulse amplitude 0.05 V, sample width 0.02 s, pulse width 0.05 s and pulse period 0.5 s. The operating conditions to record square wave voltammograms were: square wave frequency (f): 15 Hz, square wave amplitude: 0.05 V and potential step (E): 0.004 V. All DPV and SWV measurements were carried out in pH 5.0 PBS.

2.3. Electrode preparation

Before electrode modification, the GCE was carefully polished to a mirror-like surface with 0.05 μm alumina on a microcloth pad. It was then dipped in a beaker containing 0.2 M H_3PO_4 solution followed by rinsing with double distilled water to remove any adsorbed alumina particles from the electrode surface. 15 μL of gold nanourchins solution was then cast onto the cleaned GCE surface and dried under an infrared lamp. The resultant electrode was used for electrochemical studies and denoted as AuNu/GCE.

2.4. Sample preparation

A commercially available PQ tablet (Malirid, Ipca Laboratories Ltd., India) containing 7.5 mg of primaquine phosphate was finely powdered using mortar and pestle. The powder was then transferred to a volumetric flask and dissolved in double distilled water. Square-wave voltammograms were recorded (as described for the standard PQ solution) at AuNu/GCE to determine PQ in the pharmaceutical formulation by taking suitably diluted aliquot of the prepared solution (that falls within the linear concentration range) and conducting SWV analysis under similar conditions as used while conducting the concentration study. The content of PQ in the sample was calculated from the related calibration equation.

Urine samples were collected from laboratory personnel and diluted 500 times with 0.1 M PBS (pH 7.2). The dilution process helps in reducing the matrix effect. The samples were then spiked with appropriate amounts of PQ solution for volumetric analysis. The standard addition method was used to determine spiked PQ in the samples.

3. Results and discussion

3.1. Characterization of AuNu/GCE

The surface morphology of the modified electrode was explored using SEM and TEM. As shown in Fig. 2A, uniformly dispersed spherical structures with “nanothorns” emerging on their surface and having an average size of 73 nm were observed on the electrode surface. The urchin-like structure of gold nanoparticles is in accordance with the morphology of gold nanourchins reported in literature [33], which was further confirmed from the TEM image (Fig. 2B). Thus, the images indicate that gold nanourchins were successfully assembled on the surface of the electrode. Furthermore, the presence of gold (Au) on the electrode surface was confirmed by energy-dispersive X-ray spectroscopy (EDX) spectra, as depicted in Fig. 2C. A strong Au peak in the figure shows that gold nanourchins were successfully coated onto the electrode surface. Trace amounts of sodium and chlorine were also found to be present in addition to carbon and oxygen.