Sensors and Actuators B 205 (2014) 352–362

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Fabrication of aminotriazole grafted gold nanoparticles films on glassy carbon electrode and its application towards the simultaneous determination of theophylline and uric acid

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ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 28 August 2014
Accepted 28 August 2014
Available online 8 September 2014

Keywords:
Heterocyclic grafted gold nanoparticles
Potentiodynamic method
XPS
Theophylline
Uric acid

ABSTRACT

This paper describes the potentiodynamic formation of AuNPs film on glassy carbon (GC) and indium tin oxide (ITO) electrodes using aminotriazole (AT) functionalized AuNPs (AT-AuNPs) and the utilization of the resulting AT-AuNPs film for the simultaneous determination of adenosine inhibitor drug, theophylline (TP) and uric acid (UA). The potentiodynamically prepared AT-AuNPs film modified electrodes were characterized by cyclic voltammetry (CV), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and UV–vis spectroscopy. The prepared AT-AuNPs film modified electrode showed excellent electrocatalytic activity towards TP and UA. Therefore, it was successfully used for the simultaneous determination of them in a mixture. Further, the AT-AuNPs film modified electrode was effectively used for the selective determination of TP in the presence of 25-fold excess UA. Using amperometry, detection of 40 nM TP was obtained. The current responses of TP was increased linearly while increasing their concentrations from 4.0×10^{-8} M to 1.0×10^{-4} M and the detection limit was found to be 8.5×10^{-9} M (S/N = 3). The practical application of the present modified electrode was demonstrated by simultaneously determining the concentrations of TP and UA in human blood serum and urine samples.

1. Introduction

Theophylline (1,3-dimethylxanthine, TP) (Chart 1), is a member of xanthine based alkaloids and it has a stimulating effect on respiration [1]. It is widely employed as a bronchodilator drug in the management of various asthmatic conditions [1,2]. It has also been effectively used as respiratory stimulators for the treatment of infant apnea, asthmatic acute phase in children [1], symptoms of acute and chronic asthmatic condition [2], bronchospasm [3], chronic obstructive pulmonary disease [4] and emphysema [5] in adults. It has been reported that TP can cause various physiological effects, such as relaxation of gastric acid secretion and stimulation of the central nervous system [6]. The most accepted range of effective plasma TP concentration in adults is between 5 and 20 $\mu g\,mL^{-1}$ [6]. Levels below this range are usually non-therapeutic, while higher levels may cause serious toxicity. For example, when the concentration is higher than 20 $\mu g\,mL^{-1}$, it can occasionally produce serious toxicity, including vomiting, tachycardia, seizures and central nervous system excitation [7,8]. The behavioural stimulant effects of the drug appear to involve blockade of adenosine receptors [9]. On the other hand, it has been reported that TP acts as an adenosine receptor antagonist and also as a phosphodiesterase inhibitor [10]. Uric acid (UA, 2,6,8-trihydroxypurine (Chart 1)) is the main end product of purine nucleotide catabolism in human body. Serum UA was a risk factor for the oxidative stress [11], coronary heart disease [12] and elevated serum UA was associated with a risk of cardiovascular disease [13]. TP increases serum UA levels, leading to hyperuricemia in asthmatic patients [14–17]. The normal serum concentration of UA lies between 2 and 6 $mg\,dL^{-1}$ and in hyperuricemia the level goes beyond 7 $mg\,dL^{-1}$ in male and 6.5 $mg\,dL^{-1}$ in female [18]. Yamamoto et al. reported that TP increased the plasma concentrations of purine bases without decreasing urinary excretion of them in normal subjects and TP affected neither the concentration of nucleotides nor the activities of the enzymes related to purine metabolism (hypoxanthine-guanine phosphoribosyltransferase, 5′-nucleotidase, adenosine deaminase and purine nucleoside phosphorylase) in erythrocytes [16]. Further, they have concluded that the theophylline-induced purine degradation might be increased by the level of circulating UA [16]. However, the exact mechanism underlying TP induced production of UA remains

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http://dx.doi.org/10.1016/j.snb.2014.08.086
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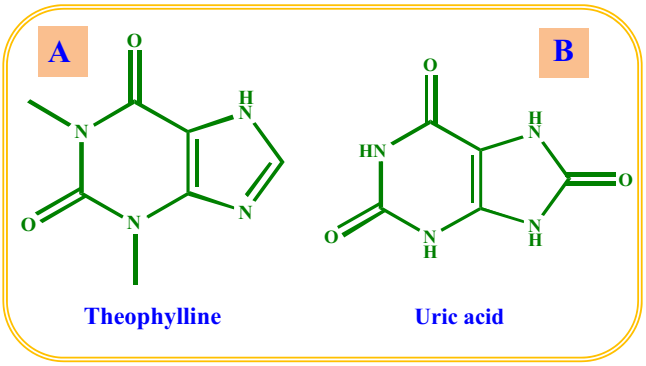

Chart 1. Structures of (A) theophylline and (B) uric acid.

uncertain. Nevertheless, an accurate determination of TP and UA in human fluids is essential for clinical point of view.

Simultaneous determination of TP and UA was reported by HPLC [19–23] and GC–MS [24] methods. It is well known that these methods are very tedious, more time consuming process and high cost. Therefore, a less expensive method with a simple procedure should be developed for the determination of TP and UA simultaneously. Electrochemical method is considered as one of the best methods among the different methods because it is less expensive, more convenient, reproducible and highly selective and sensitive. To the best of our knowledge, simultaneous determination for TP and UA by electrochemical method has not been reported so far. Hence, the objective of the present paper is to simultaneously determine TP and UA using aminotriazole grafted gold nanoparticles film modified GC electrode.

For the past two decades, modification of conducting substrates via the reduction of diazonium salts has received much attention due to its high stability and simple procedure [25]. Diazonium salt chemistry opens a potential way to produce thermally stable NPs due to the formation of metal–carbon bond [26]. Grafting of diazonium salts can be used for the stabilization of the AuNPs [26,27] and it can also be used for the modification of electrodes with AuNPs films [28–30]. Fabrication of AuNPs films on conducting substrates by potentiodynamic method has received much attention [31,32]. Enormous development in this field involves the attachment of electropolymerizable groups at the terminal NPs taking benefit of the electropolymerization process to prepare electrodes modified with electroactive NPs film [31,32]. There are few reports in the literature, regarding the embedding of AuNPs onto polypyrrole, polythiophene and polyaniline films since they can be prepared easily by both chemically and electrochemically in addition to their better conducting properties [31–35]. However, these methods involve either tedious procedure or series of steps. Further, these ligands undergo place exchange [36] and easily displaced in the presence of competitive solvents, temperature and pH [37]. Diazonium grafted AuNPs will resolve this problem due to its high stability and roughness [38]. Very recently, we have reported the synthesis of aminophenyl (AP) functionalized AuNPs (AP-AuNPs) in an aqueous medium [39] and their film on GC electrode [40]. In this paper, an attempt is made to prepare heterocyclic grafted AuNPs film by potentiodynamic method using 3,5-diamino-1,2,4-triazole (DAT) grafted AuNPs and utilization of the resulting AuNPs film for the simultaneous determination of TP and UA. It is interesting to know whether the film formation behaviour of DAT grafted AuNPs and the electrocatalytic activity of the resulting film is similar to aminophenyl AuNPs films [40] or not. Since DAT contains triazole ring and also nitrogen hetero atoms, it is expected that the sensitivity of the AuNPs film towards analytes may be enhanced via non-covalent interactions.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$), 3,5-diamino-1,2,4-triazole (DAT), theophylline (TP), uric acid (UA), ascorbic acid (AA), dopamine (DA), xanthine (XN) and caffeine (CAF) were purchased from Sigma–Aldrich and were used as received. Sodium borohydride ($NaBH_4$), sodium nitrite ($NaNO_2$) and hydrochloric acid (HCl) were purchased from Merck (India). Aminotriazole diazonium cations (ATD) were generated in situ from DAT [41]. 1 equiv. DAT was dissolved in 0.5 mol dm^{-3} HCl. To this solution, 1 equiv. cold $NaNO_2$ was added drop wise to generate the ATD in the electrochemical cell. Indium tin oxide (ITO) substrates were purchased from Asahi Beer Optical Ltd, Japan. 0.2 M phosphate buffer (PB) solution (pH 7.2) was prepared by using Na_2HPO_4 and NaH_2PO_4. All other chemicals were of analytical reagent grade and were used as received. Double distilled water was used to prepare the solutions.

2.2. Instrumentation

High resolution transmission electron microscopy (HR-TEM) images of AuNPs were obtained from a JEOL JEM 3010 operating at 200 kV. The samples were prepared by dropping 2 μL of a colloidal solution onto a carbon-coated copper grid. UV–vis spectra were recorded with a JASCO V 630 (Japan) UV–vis spectrophotometer. XPS measurements were carried out by using Shimadzu Axis 165 high performance multitechnique analysis using an Al Kα source with pass energy of 80 eV, where the pressure in the analysis chamber was lower than 133.3×10^{-8} Pa and the dwell time was 458 ms. The binding energies for identical samples were reproducible within ±0.10 eV. A survey spectrum and core-level spectra of C 1s (280–290 eV), O 1s (526–538 eV) and N 1s (396–410 eV) regions were systematically recorded. The energy scale of the instrument was calibrated by setting Au $4f_{7/2}$ = 84.00 eV and Ag $3d_{5/2}$ = 368.70 eV. The spectra were calibrated on the C 1s peak (285.0 eV). Atomic sensitivity factors are C 1s 1.0, O 1s 2.93, S 2p 1.68, N 1s 1.8, and Au $4f_{7/2}$ 9.58. Spectra were analyzed using XPS-PEAK41 software. After subtraction of a Shirley background, all spectra were fit by use of a convolution of Gaussian functions. The peak-fitting procedure used a minimum number of peaks consistent with the best fit with consideration of peak position, full width at half-maximum, and intensity. The tapping mode AFM images were recorded using a multimode scanning probe microscope (NTMDT, NTEGRA prima, Russia) with a Cantilever model NSG03-A (NTMDT, Russia) and force constant value ranges from 0.35 N m^{-1} to 6.06 N m^{-1}. Electrochemical measurements were performed in a conventional two compartment three electrode cell with a mirror polished 3 mm glassy carbon (GC) electrode as a working electrode, Pt wire as a counter electrode and a NaCl saturated Ag/AgCl as a reference electrode. All the electrochemical measurements were carried out with CHI model 634B (Austin, TX, USA) Electrochemical Workstation. For differential pulse voltammetry (DPV) measurements, pulse width of 0.06 s, amplitude of 0.05 V, sample period of 0.02 s and pulse period of 0.2 s were used. All the electrochemical measurements were carried out under nitrogen atmosphere at room temperature.

2.3. Synthesis of AT-AuNPs

All glasswares were thoroughly cleaned with freshly prepared aquaregia (3:1; HCl/HNO_3) and rinsed comprehensively with double distilled water prior to use. The AT-AuNPs were prepared as follows. First, AuNPs were prepared by adding 250 μL of 31.7 mM $HAuCl_4{\cdot}3H_2O$ solution into 25 mL of water. Then, 200 μL of 0.3 M