



# Electrochemical Biosensor consisted of conducting polymer layer on gold nanodots patterned Indium Tin Oxide electrode for rapid and simultaneous determination of purine bases



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## ARTICLE INFO

### Article history:

Received 21 September 2013

Received in revised form

18 December 2013

Accepted 20 December 2013

Available online 15 January 2014

### Keywords:

Biosensor

Adenine and Guanine

Differential pulse voltammetry

Conducting polymer nanostructures

Human serum

## ABSTRACT

The present work focused on the development of new simple method for fabrication of conducting poly(4-aminothiophenol) nanostructures layered on gold nanodots patterned indium tin oxide (ITO) electrode based on the self assembly of the monomer. This was followed by electrochemical polymerization of 4-aminothiophenol molecules. In addition, we studied the electrochemical catalytic activity of the modified electrode towards a mixture of two protein bases (Adenine and Guanine). The modified gold nanodots ITO electrode was fabricated based on thermal evaporation of pure gold metal onto ITO surface through polystyrene monolayer. Then, a monolayer of 4-aminothiophenol was self-assembly immobilized onto the gold nanodots array/ITO electrode. This was followed by electrochemical polymerization process based on cyclic voltammetry technique. The electrochemical behavior of the adenine and guanine mixture at the modified electrode was investigated based on differential pulse voltammetry technique. The results indicated that the polymer nanostructures modified gold nanodots/ITO electrode exhibited an excellent electrocatalytic activity towards the oxidation of adenine and guanine with a detection limit of 500 and 250 nM, respectively. Moreover, our finding demonstrated a linear relation between the concentration of both adenine and guanine and their oxidation current peaks ( $R=0.9953$  and  $0.9935$ , respectively). Finally, the modified electrode was successfully used to detect adenine and guanine in human serum sample. Therefore, we proposed that this biosensor could have high sensitivity for simultaneous determination of adenine and guanine in the related physiology process.

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## 1. Introduction

Adenine (A) and guanine (G) are two of the purine bases that play a vital role as essential building blocks of nucleic acids. These two bases are fundamental compounds in different biological systems, contribute in several processes such as energy transduction, metabolic co-factors and cell signaling. Abnormal change of A and G concentration is considered as indication for the deficiency of the immunity system. Thus, there is great demand for the monitoring of the variation of A and G concentration in living organisms.

Several techniques has been developed to determine the concentration of these purine bases in DNA such as HPLC and isotope dilution mass spectrometry techniques [1]. Although these techniques showed high sensitivity and selectivity, they are

time-consuming and require complicated instruments. Therefore, they are unsuitable for real time determination field.

Recently, electrochemical methods for detection of purine bases (A and G) have attracted attention due to their advantages including; fast response speed, cheap instrument, good sensitivity and selectivity [2–4]. However, the direct oxidation of A and G at the traditional bare electrodes has shown poor response with slow electron transfer, in addition to higher over potential of their oxidation that leading to a low selectivity and sensitivity [5]. Hence, numerous chemically modified electrodes such as carbon ionic liquid electrode and fullerene-C60-modified glassy carbon electrode [6,7] had been developed to widen the potential range in which oxidation of A and G occurs. While, these modified electrodes has shown improvement in oxidation signals; however, most of them has elicited complicated preparation process, high background current and leakage of modified electrodes. It is still a challenging to develop stable electrode modified material with high sensitivity and selectivity.

Different metal and semiconductor nanoparticles (that exhibit unique optical, electrochemical and catalytic properties) have been

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widely used to improve the electrode's surface area as well as its sensitivity. Therefore, it enhanced their applications in electrochemical sensors and biosensors [8–13]. Furthermore, there is growing interest in the use of gold (Au) nanoporous modified electrode due to the wide range of applications that require inertness, conductivity and/or large surface area [14]. Also, nickel oxide or zinc oxide nanoparticles modified electrodes exhibited high electrocatalytic activity towards different biological materials [15,16].

Recently, we have developed Au nanoporous layers modified electrode to change the mass transport processes from planar diffusion to thin layer character [17,18]. That could shift the potential under which the target redox active species from that required to electrolyse the interfering species that facilitate the differentiation between species that oxidize or reduce at similar potentials under planar diffusion conditions [19,20]. Moreover, conducting polymers have a wide variety of applications such as sensors, cathode material of a lithium secondary battery, electrocatalysts and microelectronic devices [21,22] due to their good electrochemical properties. In addition, conducting polymers coating metal nanostructures have been of great interest due to the ability of them to generate additional electro-catalytic sites as well as the probable electronic interactions between metal nanostructures and groups in the polymer [23,24]. Recently, modified Au nano-particles based on self-assembly is promising for the construction of nano-devices and nano-biosensors [25].

Self-assembly of 4-Aminothiophenol (ATP) onto bulk Au electrode has been used for selective determination of dopamine and ascorbic acid [26]. However, the bulk electrodes bear disadvantages; slow electron transfer, low sensitivity, and poor selectivity [27]. In the recent years, ATP has attracted significant attention in producing assembly layer onto nanoparticles via covalent or electrostatic interactions [28]. The difference in reactivity between the thiol and amine ends of ATP has been effectively utilized to design molecular assemblies leading to unique morphologies and chemical treatments [29].

On the contrary, previous studies have reported fabrication of conducting polymer coating Au nanostructures based on disperse Au nanoparticles into thin film such as PATP (poly(4-aminothiophenol)) over the surface of different electrodes [30–33]. Up to our knowledge, the present work is the first to report fabrication of a newly polymer nanostructures modified electrode based on thermal evaporation of pure gold metal onto ITO surface through monolayer of polystyrene. These Au nanodots acted as template for uniform immobilization of ATP molecules based on self-assembly of a monolayer of ATP onto Au nanodots/ITO electrode. Due to the selectivity interaction between ATP molecules and Au surface, ATP molecules could be attached solely to the surface of Au nanodots. Thus, ATP will be converted into PATP based on electro-chemical polymerization of the ATP film. The electro-chemical oxidation behavior of A and G was investigated by PATP/Au nanodots/ITO electrode. In summary, the performance of the fabricated electrode such as; sensitivity, linear range and selectivity, has been evaluated and discussed. Also, the applicability of this modified electrode has been demonstrated by determining G and A bases in real samples.

## 2. Experimental

### 2.1. Materials

ATP, A, G, human serum (HS) and phosphate-buffered saline (PBS) (pH 7.4, 10mM) were purchased from Sigma (St. Louis, MO, USA). The other chemicals used in this study were obtained commercially as reagent grade. All solutions were prepared with de-ionized water (DIW).

### 2.2. Instruments

Electrochemical polymerization of ATP into PATP and all electrochemical experiments were performed using a potentiostat (CHI-660, CH Instruments, USA) controlled by general-purpose electrochemical system software. A homemade three-electrode system consisted of a nano-PATP/Au nanodots/ITO substrate as a working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl as the reference electrode. In addition, the synthesis of nano-PATP/Au nanodots/ITO was characterized by Fourier transform infrared spectrophotometer (FT-IR) using Nicolet is10 FT-IR spectrometer (Thermo Scientific). The morphologies of Au nanodots/ITO as well as nano-PATP/Au nanodots/ITO were analyzed by a scanning electron microscope (SEM) (ISI DS-130 C, Akashi Co., Tokyo, Japan).

### 2.3. Fabrication of Au nanodots/ITO substrate

Au nanodots modified ITO substrates have been fabricated by using monolayer of polystyrene (PS) as an evaporation mask based on the thermal deposition of Au layer onto ITO substrate. The PS masks that has been prepared by the following method; 7  $\mu$ L of PS particles with a diameter of 500 nm (10 wt % aqueous solution) mixed with a surfactant (Triton-X and methanol 1:400 v/v) in a ratio of 1:1 was applied onto the ITO substrate. Different spin speeds (500, 1000 and 1500 rpm) for 1 min were used. The substrate was then left to dry in the spin coater with a covered lid to maintain a consistent drying ambient and evaporation rate [34]. Then, an Au layer (50 nm) was thermally evaporated through the deposited PS nano-sphere mask. Fifty nm thickness of the Au layer has been selected to fill the spaces between the PS nano-spheres and to obtain the triangle nanostructures. This resulted in a uniform enhancement of the electro-chemical activity. In addition, evaporation of thicker Au layer will not change the morphology of the resulted triangle nano-structures. After dissolution of the PS mask in chloroform, an array of ordered triangle Au nanostructures was left onto the ITO substrate.

### 2.4. Fabrication of PATP/Au nanodots/ITO electrode

A monolayer of 4-aminothiophenol was self-assembled on Au nanodots/ITO substrates by immersing the substrates in 2 mM of 4-aminothiophenol ethanol solution for 2 hrs, then the fabricated ATP-modified substrates were rinsed thoroughly by DIW and ethanol successively, then dried under  $N_2$  gas. Fabrication of nano-PATP/Au nanodots/ITO was achieved by applying cyclic voltammetry (CV) technique to ATP self-assembled monolayer in 0.5 M  $HClO_4$  aqueous solution from 0.0V to 1.0V for 15 cycles at scan rate of 50 mV/sec.

## 3. Results and Discussion

### 3.1. Characterization of PATP/Au nanodots/ITO electrode

Herein, the uniform distribution of Au nanodots over ITO has been used as an effective template to develop nano-PATP/Au nanodots/ITO substrates, because of the selectivity interaction between ATP molecules and Au surface. ATP molecules could be attached only on the surface of Au nanodots by covalent bonds between thiols ends of ATP molecules and Au nanodots (Scheme 1). Different spin speeds were used to achieve a uniform monolayer over large area of the substrate. Fig. 1a showed that multilayer film was obtained by applying spin speed of 500 rpm; while, spin speed of 1500 rpm resulted in non-compacted monolayer film (Fig. 1b). Whilst, a closed-compacted monolayer film (Fig. 1c) was obtained with spin speed of 1000 rpm speed.

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