



Electrochemical deposition of gold nanoparticles on reduced graphene oxide modified glassy carbon electrode for simultaneous determination of levodopa, uric acid and folic acid



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ABSTRACT

A novel assembly of graphene oxide (RGO), gold nanoparticles and 2-(3,4-dihydroxy phenyl) benzothiazole (DHB) modified glassy carbon electrode (DHB/AuNPs/RGO/GCE) was successfully fabricated by chemical and electrochemical deposition of gold nanoparticles at RGO/GCE and characterized by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques. This sensor was applied to simultaneous determine levodopa (LD), uric acid (UA) and folic acid (FA). It was observed that an electrochemical deposition of gold nanoparticle has higher electrocatalytic activity for the oxidation of LD, UA and FA compared with chemical deposition. Square wave voltammograms of these compounds showed three well defined and fully resolved anodic oxidation peaks with large current at DHB/AuNPs/RGO/GCE. The proposed electrode was successfully applied for determination of LD, UA and FA in some real samples (such as tablet of Madopar, urine and human blood serum) by the standard addition method.

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1. Introduction

Some biological compounds have an overlapping oxidation potential on the bare electrodes. Modification of electrodes using nanomaterial and redox modifiers due to reduction of the overpotential essential for electrochemical reaction is an interesting field in electroanalytical chemistry. A precise modification of the surface structure on a nanometer-scale, namely self-assembly of organo-sulfur compounds on gold surface, has been employed in the fabrication of sensors and biosensors.

It was known that patients with Parkinson's disorder have low concentration of dopamine (DA) in their brains [1,2]. Levodopa (LD) is a vital catecholamine in a biological system which is used to reduce the symptoms of Parkinson's disease by increasing of DA in the brain. But if LD is taken at high dosages, some effects of systemic DA can appear. Several methods for determination of LD have been described such as HPLC [3], capillary electrophoresis [4,5] and spectrophotometry [6,7]. Some electrochemical methods for determination of LD have also been reported due to LD is an

electroactive component with two OH groups (Scheme 1) which are the active sites for oxidation of levodopa [8–14].

Monitoring of uric acid (UA), a product of the metabolic breakdown of purine nucleotides, is essential because high blood concentrations of uric acid can lead to several diseases such as gout and hyperuricaemia [15]. Several technologies have been employed for UA analysis such as chemiluminescence [16], enzymatic-spectrophotometric [17] and electrochemical methods [18–21].

Folic acid (FA) or vitamin B9 can help to produce healthy cells and is an agent for cancer prevention by antioxidant activity [22]. Its biological importance comes from the conversion of tetrahydrofolate into dihydrofolic acid in the liver. Because of being electro active of FA, some electrochemical methods have been used for its determination [23,24].

As mentioned above, LD, UA and FA are compounds of great biomedical interest. LD and UA can cause hyperuricaemia and gout diseases. When production of UA increases because of some disease like kidneys deficiency, levels of UA build up in the blood. These conditions lead to hyperuricemia. Then gout can happen as a result of hyperuricemia [25]. LD, UA and FA have similar oxidation potential and coexistence in pharmaceutical preparations, Then UA and FA show serious interference for the determination of LD [26].

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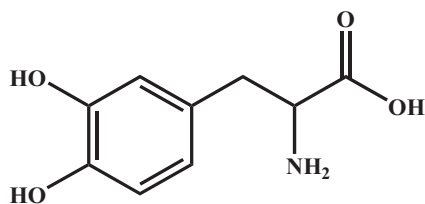
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These are the reasons that the development of a new method for the simultaneous determination of LD, UA and FA has appeared to be of great importance. It is very necessary to control the content of LD, UA and FA in real samples to achieve better medicinal effect and lower toxicity. Therefore, it is important to introduce a simple, low cost, fast, sensitive and selective detection method for the simultaneous determination of these substances.

Recently, modified electrodes with graphene–metal nanoparticles composite have attracted extensive attention in many fields because of their high electron conductivity and good biocompatibility [27,28]. Graphene, a one-atom-thick sp^2 bonded carbon sheet, has high surface area, excellent electrical conductivity and electron mobility at room temperature, which can be used to fabricate novel sensors for virtual applications.

Gold nanoparticles (AuNPs) have found many applications in biosensors due to their excellent characteristics such as high catalytic activity, huge surface area, small dimensional size, effective mass transport, and having compatible environment [29,30]. Modification of electrode with reduced graphene oxide (RGO) and AuNPs could increase the surface area of electrode; enhance optical, electronic and catalytic properties. These modified electrodes can react with organo-sulfur compounds to form S-Au covalent bonds resulting in monolayer molecular assemblies. These types of modifications can greatly increase the immobilized amount of S-functionalized compounds and enhance the stability of S-Au bond and self-assembled monolayer (SAMs). DHB has s-functional group that can easily adhere to AuNPs and produce S-Au bond for the formation of self-assembling and modification of the electrode surface. Also, DHB is a catechol with two OH groups that are the active sites for oxidation. Therefore, we used the combination of AuNPs and RGO to increase the surface area of electrode and immobilize more DHB on the electrode surface.

Several works have been reported for electroanalytical determination of LD [8,14,31–34]. Babaei et al. fabricated a carbon paste electrode modified with ionic liquid, multi-walled carbon nanotubes and cobalt hydroxide nanoparticles for simultaneous determination of levodopa and serotonin [8]. Wang et al. used reduced graphene oxide modified glassy carbon electrode (RGO/GCE) for determination of LD in the presence of carbidopa with a detection limit of 0.8 μ M [31]. Yi et al. [32] used RGO for determination of LD in the presence of ascorbic acid and uric acid. Weichao et al. used gold nanoparticle /MWCNT/L-cysteine in modification of glassy carbon electrode for determination of LD [33]. Hu et al. used gold nanoparticle and carbon nanotube for modification of pyrolytic graphite electrode for determination of LD in the presence of uric acid and ascorbic acid [34]. They obtained a detection limit of 0.05 nM for LD. Some of these methods have short linear range [31–34] and some of them have high detection limit [8,31,32]. In the present work we used combination of RGO, AuNPs and DHB for detection of LD. Due to unique properties of RGO and AuNPs, lower detection limit and wider linear range were obtained compared with other similar sensors. Also to the best of our knowledge, there have been no report on the fabrication of SAMs by synthesized RGO, gold nanoparticles and 2-(3,4-dihydroxy phenyl) benzothiazole (DHB) for simultaneous detection of LD, UA and FA.



Scheme 1. Structure of levodopa.

In this work, AuNPs were prepared by using two methods: chemical and electrochemical methods. The modified electrode was characterized by cyclic voltammetry (CV), scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) techniques. Modification of electrode by electrochemical deposition of AuNPs shows more advantages in comparison with modification by chemical method. We also report the application of DHB/AuNPs/RGO/GCE as a new electrode for the electrocatalytic determination of LD in phosphate buffer solution. The analytical performance of the modified electrode has been also evaluated during LD quantitation in the presence of UA and FA. The applicability of the modified electrode has successfully demonstrated by voltammetric determination of LD in real samples.

2. Experimental

2.1. Apparatus and chemicals

Electrochemical measurements were performed with a potentiostat/galvanostat Autolab model 302 N (Eco Chem, Utrecht, Netherlands) and a NOVA 1.7 software at laboratory temperature (25 ± 1 °C). An Ag/AgCl (KCl, sat) electrode, a platinum wire and Glassy carbon electrode (Azar electrode Co., Urmia, I.R. Iran, 2 mm diameter) modified with RGO, Au nanoparticle and DHB self-assembled monolayer (DHB/AuNPs/RGO/GCE) were used as reference, auxiliary and working electrodes, respectively. pH measurements were carried out with a Metrohm model 691 pH/mV meter. All solutions were prepared with doubly distilled water. LD, UA, FA and other reagents were analytical grade (Merck). The buffer solutions (0.1 M) were prepared from phosphoric acid and sodium hydroxide solutions in the pH range of 4.0–10.0.

2.2. General procedure for the synthesis of 2-(3,4-dihydroxy phenyl) benzothiazole

A mixture of 2-aminothiophenol (1.2 mmol), 3,4-dihydroxybenzaldehyde (1 mmol) and $Al(HSO_4)_3$ (0.02 g) was heated at 80 °C for 10 min. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the mixture was cooled to room temperature, dissolved in acetone followed by addition of water, the solid product was appeared. The product was re-crystallized in hot ethanol. FT-IR (ATR, neat), $\nu = 3478(OH)$, 1470 (C=N), 1264 (C–O), 1175(C–O), 1364, 1200, 1071, 1055, 765, 721 cm^{-1} . 1H NMR (400 MHz, Acetone- d_6): δ : 6.98 (d, $J = 8.4$ Hz, 1H), 7.38 (t, $J = 7.2$ Hz, 1H), 7.51 (m, 2H), 7.67 (d, $J = 2$ Hz, 1H), 7.96 (d, $J = 8.0$ Hz, 1H), 8.02 (d, $J = 8.0$ Hz, 1H), 8.50 (s, 1H), 8.69 (s, 1H).

2.3. Preparation of electrode

Graphene nanosheets were prepared by oxidizing graphite using a new method [35]. For the reduction, GO suspension in purified water (150 mg/50 mL) was added to 50 μ L of hydrazine solution (98%) with 200 μ L of ammonia solution (30%). After refluxing at 90 °C for 12 h, solution was cooled down. Subsequently, solution was centrifuged and precipitates were washed with deionized water and then dried at 60 °C in vacuum for 24 h [36]. SEM was applied for characterizing of RGO (Fig. 1) which can easily adhere to a GCE surface via interaction between graphene and glassy carbon [37]. Two following methods were used for modification of RGO/GCE with AuNPs.

2.3.1. Chemical method

In the chemical method, AuNPs were prepared according to Lee and Mesial's method with sodium citrate reduction of $HauCl_4$ [38]

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