Sensors and Actuators B 145 (2010) 643–650

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Simultaneous determination of ascorbic acid, dopamine and uric acid using polystyrene sulfonate wrapped multiwalled carbon nanotubes bound to graphite electrode through layer-by-layer technique

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ARTICLE INFO

Article history:
Received 16 December 2009
Received in revised form 4 January 2010
Accepted 6 January 2010
Available online 15 January 2010

Keywords:
Polystyrene sulfonate
Multiwalled carbon nanotubes
Layer-by-layer technique
Ascorbic acid
Dopamine
Uric acid

ABSTRACT

A promising electrochemical system is fabricated using layer-by-layer (LBL) technique on graphite electrode, by positively charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged multiwalled carbon nanotubes (MWCNTs) wrapped with polystyrene sulfonate (PSS) through electrostatic interaction, for the simultaneous determination of ascorbic acid (AA), dopamine (DA) and uric acid (UA). Solubility of MWCNTs in water was increased by using linear polymer PSS. The PSS wrapped MWCNTs modified electrodes were characterized by electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) and chronoamperometric techniques. The modified electrode exhibits superior electrocatalytic activity towards AA, DA and UA than the bare graphite electrode. The three separated anodic peaks were obtained at 192, 123 and 315 mV between AA–DA, DA–UA and AA–UA respectively in CV and corresponding separated anodic peaks were 210, 119 and 329 mV in DPV respectively. No electrode fouling was observed during all the experiments and good stability and reproducibility was obtained for simultaneous determination of AA, DA and UA.

1. Introduction

Dopamine (DA) plays an important role in the function of central nervous, renal, hormonal and cardiovascular systems [1]. It is of great clinical importance to measure the DA level in extracellular fluid to monitor neurotransmission processes and diagnose Parkinson's disease. There is an intense investigation in the development of methods for DA quantification in blood and biological fluids. Electrochemical methods have proven to be rapid, simple and sensitive in the determination of neurotransmitters. However, an overlapping voltammetric response has been observed because the oxidation of DA at bare electrodes occurs along with the oxidation of AA and UA in biological tissues [2–4]. Thus, it is a challenge to separate the oxidation peaks of AA, DA and UA from each other in electrochemical analysis. UA is an important analyte in clinical field. In a healthy human being, the typical concentration of UA in urine is in millimolar range (~2 mM), whereas in blood it is in the micro-molar range (120–450 μM) [5,6]. Abnormalities of UA level indicate symptoms of several diseases, such as gout, hyperuricaemia and Lesch-Nyhan syndrome [7]. Ascorbic acid (vitamin C) is a water-soluble substrate present in a wide number of foods such as, fruits and vegetables. AA is also added to foodstuffs as an antioxidant for stabilization of color and aroma, as well as prolonging the life of commercial products [8]. Due to the presence of ascorbate in the mammalian brain, it plays an important role in bioelectrochemistry, neurochemistry and clinical diagnostics applications. It is also necessary for the formation of collagen and has been used for prevention and treatment of common cold, scurvy and cancer [9].

Since two decades carbon nanotubes (CNTs) have been gaining popularity due to their unique properties such as electronic, metallic and structural characteristics [10]. CNTs have outstanding ability to mediate fast electron transfer kinetics for a wide range of electroactive species and show electrocatalytic activity towards biologically important compounds such as NADH [11], dopamine (DA), ascorbic acid (AA) and uric acid (UA) [12], H_2O_2 [13], morphine [14] and DNA [15,16]. CNTs mainly serves as transducers, they have been used to facilitate immobilization of biological molecules and for biosensor applications [17,18]. They are insoluble in most of the solvents but can be temporarily dispersed in

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doi:10.1016/j.snb.2010.01.011

DMF [19,20], acetone [21], etc., the dispersion was then cast on to the electrodes surface, later solvent was evaporated. The resulting CNTs layer on electrode surface was mechanically and electrically unstable; this limits their applications in sensors. Several strategies have been proposed to dissolve CNTs in various solvents, among them polymer wrapping is one of the method. Wang et al. [22] reported the solubilisation of CNTs in nafion solution and developed a glucose biosensor based on the nafion-solubilised CNTs. Zhang et al. have constructed dehydrogenase biosensor based on solubilisation of CNTs in chitosan solution [23]. In both the systems, electrode modification was done by casting CNTs on the electrode surface. This method could not allow the control all the properties of CNTs. Zhao and Ju [24] have reported glucose sensor based on poly(diallyldimethylammonium chloride) (PDDA) wrapped with CNTs and constructed stable and uniform multilayers. O'Connell et al. [25] reported the solubilisation of CNTs in water by non-covalently associating them with linear polymer such as polyvinyl pyrrolidone (PVP) or polystyrene sulfonate (PSS). The authors have demonstrated that the polymer was uniformly wrapped around the tubes rather than associated with side walls at various points as random coils.

AA, DA and UA are electroactive compounds with a very similar electrochemical properties and they will oxidized at nearly same potential with poor sensitivity at unmodified electrodes. Therefore simultaneous determination of AA, DA and UA is a major goal in modifying the electrodes. Various modified electrodes have been constructed. A working electrode coated with ion-exchange membrane such as nafion was proposed to avoid electrode surfaces from interferences [26,27]. However, this kind of modified electrodes suffers from slow response due to low diffusion coefficient of analytes through the films. Detection sensitivity of DA and UA in presence of high concentration of AA was improved by nafion coated clay-modified electrode [28]. The disadvantages of ion-exchange membrane modified electrodes include non-uniform thickness and poor reproducibility due to solvent evaporation method used in the film preparation. Electro-polymerization of conducting polymers can be used to prepare polymer films with uniform and controllable thickness on the electrode surface. Due to their high selectivity, various polymer-modified electrode have been used for determination of AA, DA and UA [29–31]. Apart from CNTs, nanogold modified carbon ionic liquid electrode [32], Fe_3O_4 nanoparticles [33], gold nanoparticles/choline composites [34] and Pd nanoparticles [35] have also been used for determination of AA, DA and UA in different combinations.

The layer-by-layer (LBL) technique has become the prime choice for fabrication of nanostructured films and can be achieved in a straightforward, low-cost manner [36]. Using LBL technique a wide diversity of materials may be employed and film fabrication is performed under mild conditions, which is particularly important for preserving activity of biological substances. This technique is based on alternate electrostatic adsorption of the negatively/positively charged species. So far, the technique has been successfully used for a wide range of biomolecules such as proteins [37], NADH [38], etc. Zhang et al. [39] reported the layer-by-layer technique for selective determination of DA in presence of AA based on electrostatic interaction between the positively charged PDDA and negatively charged –COO– functional group introduced CNTs. Qu et al. [40] developed amperometric biosensor for choline on layer-by-layer assembled carbon nanotubes and polyaniline multilayer films. By using this technique homogeneous and stable MWCNTs and polyaniline (PANI) multilayer films were constructed on glassy carbon electrode. During same year Chen et al. [41] studied electrocatalytic oxidation and sensitive detection of cysteine using layer-by-layer technique. CNT modified electrode was fabricated through LBL electrostatic deposition of positively charged PDDA and negatively charged shortened MWCNTs on glassy carbon electrode. Kong et al. [42] have fabricated multilayer films MWCNTs with molecular recognition function on glassy carbon electrode with lower capacitive background current using LBL method.

During these days, LBL technique has attracted much attention of researchers because it involves simple procedure, much faster, versatile and eco-friendly (conducted in aqueous medium). In addition, the roughness, thickness and porosity of the film can be tuned at the molecular level by adjusting experimental parameters such as pH, ionic strength and polyelectrolyte concentration. In the present study we have formed layer-by-layer assembly of negatively charged MWCNTs wrapped PSS and positively charged PDDA through electrostatic interaction. The layer-by-layer assembly of PDDA and MWCNTs wrapped PSS has been successfully used to develop the simultaneous detection of AA, DA and UA.

2. Experimental details

2.1. Reagents

MWCNTs, PDDA (M_w: 200,000–350,000), PSS (M_w: 70,000), AA, DA and UA were purchased from Sigma–Aldrich and used as received. MWCNTs were purified, shortened and –COO– introduced by refluxing in conc. HNO_3 for 5 h, filtered washed with double distilled water until filtrate became neutral and finally dried under vacuum. Phosphate buffer solutions (PBS) were prepared from stock solution of 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4. pH was adjusted using 0.5 M HCl and 0.5 M NaOH. All other chemicals used were of analytical reagent grade unless otherwise mentioned and used without further purification. All solutions were prepared with double distilled water. The electrolyte solutions were deoxygenated by bubbling ultra-pure nitrogen for at least 10 min. And during electrochemical experiments nitrogen blown over the solution surface to make homogeneous mixture.

2.2. Preparation of modified graphite electrode

An electrode was fabricated by inserting 6 mm diameter graphite cylinder in the hole of a Teflon bar with same internal diameter; contact was made with copper wire through the centre of Teflon bar. The electrode was polished with emery papers of different grades, i.e., 1000, 800, 6/0, 4/0, and finally with 2/0 until a mirror shining surface was obtained and finally rinsed with double distilled water in an ultrasonic bath for 6 min. MWCNTs (2 mg/ml) were solubilised in PSS (2 mg/ml) and then ultrasonicated for 15 min to give MWCNTs dispersion, which was then incubated at 50 °C for 24 h. The polished graphite electrode was first dipped in PDDA (1%, w/v) containing 0.5 M NaCl for 25 min. The electrode was carefully rinsed with distilled water to remove the excess and loosely held polymer material and then dried with nitrogen gas. The positively charged, PDDA modified graphite electrode was immersed in MWCNTs/PSS solution for 25 min. This procedure was repeated five times to obtain Gr/(PDDA–[PSS-MWCNTs])$_5$ graphite electrode. Hereafter the modified electrode is referred as Gr/(PDDA–[PSS-MWCNTs])$_5$ graphite electrode. The addition of 0.5 M NaCl to PDDA solution gave a uniform multilayer growth, since the presence of salts clearly increases the amount of polyelectrolyte deposition [43]. The modified electrode was washed and stored in phosphate buffer solution pH 7.

2.3. Electrochemical measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were carried out with Versa stat 3 (Princeton applied research, USA) and differential pulse voltammetry (DPV) and chronoamperommetry (CA) experiments were performed with EA-201 Electro analyzer (Chemilink Systems) work