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Simultaneous voltammetric detection of dopamine and uric acid at their physiological level in the presence of ascorbic acid using poly(acrylic acid)-multiwalled carbon-nanotube composite-covered glassy-carbon electrode

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Abstract

The use of poly(acrylic acid) (PAA)-multiwalled carbon-nanotubes (MWNTs) composite-coated glassy-carbon disk electrode (GCE) (PAA-MWNTs/GCE) for the simultaneous determination of physiological level dopamine (DA) and uric acid (UA) in the presence of an excess of ascorbic acid (AA) in a pH 7.4 phosphate-buffered solution was proposed. PAA-MWNTs composite was prepared by mixing of MWNTs powder into 1 mg/ml PAA aqueous solution under sonication. GCE surface was modified with PAA-MWNTs film by casting. AA demonstrates no voltammetric peak at PAA-MWNTs/GCE. The PAA-MWNTs composite is of a high surface area and of affinity for DA and UA adsorption. DA exhibits greatly improved electron-transfer rate and is electro-catalyzed at PAA-MWNTs/GCE. Moreover, the electro-catalytic oxidation of UA at PAA-MWNTs/GCE is observed, which makes it possible to detect lower level UA. Therefore, the enhanced electrocatalytic currents for DA and UA were observed. The anodic peak currents at ∼0.18 V and 0.35 V increase with the increasing concentrations of DA and UA, respectively, which correspond to the voltammetric peaks of DA and UA, respectively. The linear ranges are 40 nM to 3 μM DA and 0.3 μM to 10 μM UA in the presence of 0.3 mM AA. The lowest detection limits (S/N = 3) were 20 nM DA and 110 nM UA.

Keywords: Poly(acrylic acid)-multiwalled carbon-nanotubes composite; Dopamine; Uric acid; Ascorbic acid; Simultaneous determination

1. Introduction

Dopamine (DA) is one of the most important neurotransmitters which play vital role in the function of the central nervous, renal, hormonal and cardiovascular system (Wightman et al., 1998; Liu et al., 2005). Low levels of DA may cause neurological disorders such as schizophrenia and Parkinson's disease (Mo and Ogorevc, 2001). Therefore, it is of great clinical importance to measure DA level in the extracelluar fluid in order to monitor neurotransmission process and diagnose Parkinson's disease. Electrochemical methods have been proven to be rapid, simple and sensitive in the determination of DA. The significant problem encountered with the detection of DA arising from the low concentration levels of DA (in the range of several tens of nanomolars) and the primary interference from ascorbic acid (AA, 0.2–0.4 mM), which largely co-exists with DA and has overlapping voltammetric response at bare electrodes. The catalytic oxidation of AA by oxidized DA is another major interference in the detection of DA. Thus, it is of importance to distinguish DA from AA or elimination of the interference of AA by using suitable coating film (Nowall and Kuhr, 1995; Popa et al., 1999; Liu et al., 2006a). For this purpose, various modified electrodes have been constructed. For example, DA can be selectively detected in the presence of AA at electrodes modified with titanate-nanotube (Liu et al., 2006a), boron-doped diamond (Nowall and Kuhr, 1995; Poh et al., 2004), polyelectrolyte-layer (Ciszewski and Milczarek, 1999), monolayer (Malem and Mandler, 1992; Raj and Osaka, 2001; Lin and Gong, 2004), carbon-nanotube (CNT) (Zhang et al., 2005) and negatively charged polymer (Kawagoe et al., 1993).

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Uric acid (UA) and other oxypurines are main final products of purine metabolism in the human body. Disorder of purine biosynthesis and/or purine catabolism, such as gout, hyperuricemia, and Lesch–Nyhan syndrome are generally considered due to the abnormal concentrations of UA dissolved in human urine and/or blood (Hall et al., 1967; Ullman et al., 1982). Because AA and UA are co-existent in blood and urine, it holds great importance to selectively detect UA in the presence of AA. The oxidation potentials of UA and AA at bare electrodes are so near that it is difficult to separate their voltammetric peaks. Several electrochemical techniques including electrodes modified with uricase (Uchiyama et al., 1991; Markas et al., 1994; Nakaminami et al., 1999) diamond-film (Popa et al., 2000) and clay-layer (Zen and Chen, 1997) have been developed for the detection of UA. Simultaneous determination of AA and UA was also reported (Zare et al., 2005). Recently, there is intense investigation in the development of methods for the detection of DA and UA (Zen and Chen, 1997; Kang and Lin, 2006; Lin and Gong, 2006; Liu et al., 2006; Zeng et al., 2006; Salimi et al., 2006), considering the co-existence of DA and UA as well as excess of ascorbic acid in extracellular fluids. However, these methods lack of sensitivity. Thus, it remains a challenge to separate the oxidation peaks of AA, DA and UA from each other at electrodes. Although the simultaneous determination of AA, DA and UA at polymer-modified electrodes was reported (Aguilar et al., 2004; Zare et al., 2006; Li and Lin, 2006; Safavi et al., 2006); it may be difficult in the use of these methods for the determination of DA and UA in biological fluids due to suffering from losing sensitivity (the detection limit was reported to be several micromolars) (Li and Lin, 2006; Lin and Gong, 2006; Liu et al., 2006; Zeng et al., 2006). In most cases, a simple polymer or polymer-complex layer was coated onto the electrode surface, which play the role of separating the voltammetric peaks other than showing electrocatalytic activity to these interested species. Obviously, further efforts are needed to develop simple, selective, sensitive and simultaneous detection of DA and UA at their physiological level.

In order to utilize the excellent properties of CNTs for bioanalytical applications, it is prerequisite to modify the nanotubes without damaging their intrinsic structures. CNTs-modified electrodes were previously prepared by coating CNTs which are suspended in organic solvents for biosensors applications (Britto et al., 1996), however, the reproducibility and the operational liftetime of those modified electrodes are not satisfactory. Non-covalent functionalization of CNTs holds great promise, because it does not disrupt the large π-electronic surface, and thus it can open the possibility to organize CNTs into 3D networks. Recent electrochemical studies have shown that CNTs are capable of promoting certain types of electron-transfer reactions (Baughman et al., 1999) to minimize electrode surface-fouling, and to enhance electrocatalytic activity (Banks et al., 2004). It was reported that GCEs modified with multiwalled carbon-nanotubes (MWNTs) showed a remarkable decrease in the over-potential for nicotinamide adenine dinucleotide (NADH) oxidation and also circumvented NADH surface-fouling effects (Musameh et al., 2002; Zhang et al., 2004; Liu et al., 2006b). It is known that the microenvironment of CNTs was changed after they are dispersed in polymer, because the inter-nanotube van der Waals forces are decreased; accordingly, the solubility of CNTs in water is obviously improved. Thus, a homogeneous, stable CNTs aqueous dispersion can be obtained. In particular, CNTs-polymer composites are popular materials used for sensing applications. Solubilization of CNTs with chitosan or Nafion was used for the preparation of amperometric biosensors (Wang et al., 2003; Zhang et al., 2004). We recently reported on the non-covalent modification of MWNTs using poly(acrylic acid) (PAA); the resultant PAA-MWNTs composite exhibits interesting spectroscopic properties (Liu et al., 2006c) and retains the electrocatalytic activity of pristine nanotubes (Liu et al., 2006b).

We present here a sensitive voltammetric approach for the detection of DA and UA in the presence of AA utilizing PAA-MWNTs composite-covered glassy-carbon electrode (PAA-MWNTs/GCE). The PAA-MWNTs composite has the advantage of carbon-nanotube with a high surface area and good affinity from PAA for DA and UA adsorption. The PAA-MWNTs/GCE shows ability of depression of the oxidation peak of AA and higher electrocatalytic activity to DA. Moreover, PAA-MWNTs/GCE can catalyze the electro-oxidation of UA, which makes it possible to detect lower level UA. This is the first time to report the reversible redox-reaction of UA. Therefore, taking the advantages of sensitivity and simplicity, the simultaneous voltammetric measurement of physiological level DA and UA in the presence of a high AA content was achieved.

2. Experimental section

2.1. Reagents

Poly(acrylic acid) (PAA, MW: 200 000), dopamine (DA), ascorbic acid (AA) and uric acid (UA) were bought from Wako Chemical Co. (Tokyo, Japan). Multiwalled carbon-nanotubes (MWNTs) were obtained from Wako Chemical Co. (Tokyo, Japan) and used as received. All the reagents were of the highest grade available and used without further purification.

2.2. Preparation of PAA-MWNTs composite

In a general procedure, ∼20 mg pristine MWNTs were dispersed in a 20-mL PAA solution (1 g/L) with ultrasonication for at least 40 min. A little precipitate (it may contain catalyst and segregated MWNTs particles) was removed by filter. PAA-MWNTs composite was obtained by freeze-drying the filtrate. A TGA analysis shows that the ratio of PAA and CNTs is normally 1:1 in PAA-MWNTs composite. The resulting PAA-MWNTs composite was re-dissolved in water under stirring to obtain a uniform black aqueous suspension in which contains 2 g/L composite.

2.3. Fabrication of PAA- or PAA-MWNTs composite film-covered glassy-carbon electrode

Glassy-carbon disk electrode (GCE, 3 mm in diameter) was used to fabricate the modified electrode. GCE was first polished on finer sandpaper, further followed by alumina slurry