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Sensitive and selective determination of L-tryptophan at physiological pH using functionalized multiwalled carbon nanotubes–nanostructured conducting polymer composite modified electrode

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ABSTRACT

This paper describes the sensitive and selective determination of L-tryptophan (Tryp) in the presence of ascorbic acid (AA) and paracetamol (PA) at physiological pH using the acid functionalized multiwalled carbon nanotubes–nanostructured 5-amino-2-mercapto-1,3,4-thiadiazole composite modified glassy carbon electrode (GC/OD/FMWCNTs/p-AMT). The acid functionalized multiwalled carbon nanotubes were covalently attached on glassy carbon (GC) electrode using 1,8-ocatane diamine (OD) as a linker and dicyclohexylcarbodiimide as a coupling agent. Subsequently, a thin layer of AMT was deposited on FMWCNTs modified GCE by potentiodynamic method. The GC/OD/FMWCNTs/p-AMT electrode shows an excellent electrocatalytic activity towards Tryp by enhancing its oxidation current enormously when compared to bare GC and GC/p-AMT electrodes. Further, the GC/OD/FMWCNTs/p-AMT electrode was successfully used for the simultaneous and selective determination of Tryp in the presence of major interferents AA and PA. The selective determination of Tryp in the presence of 1000-fold excess of AA and 500-fold excess of PA was achieved. The current response of Tryp was increased linearly while increasing the concentration of Tryp from 100 to 900 nM. The amperometric current responses of Tryp were increased linearly while increasing the concentration of Tryp in the range of 25–300 nM and a detection limit was found to be 0.54 nM ($S/N = 3$). Finally, the practical application of the present method was successfully demonstrated by determining the concentration of Tryp in blood serum samples.

1. Introduction

L-Tryptophan or (S)-2-amino-3-(1H-indol-3-yl)-propionic acid (Tryp), is an essential amino acid, needed for a normal growth and maintain the positive nitrogen balance in our body [1]. It consumes in human body from daily intake. This compound is mostly available in chocolates, egg and milk. Tryp involves two main catabolic routes. In tryptamine pathway, it gives 5-hydroxy-tryptophan in the presence of tryptophan hydroxylase. The 5-hydroxy-tryptophan further gives the important neurotransmitter serotonin, which involves improving mood and mental health [2]. Subsequently, serotonin has been converted into a neurohormone melatonin, which is used to improve sleep. In kynurenine pathway, it gives niacin. The improper metabolite of Tryp leads to abnormal levels of melatonin and serotonin. Thus, the brain serotonin level depends on the Tryp level in our body. The improper metabolite of Tryp causes hallucination, delusions, depression, Alzheimer's and Parkinson's diseases [3,4]. Hence, the sensitive and selective determination of Tryp is highly essential. Several methods have been reported for the determination of Tryp in biological and pharmaceutical samples such as high-performance liquid chromatography [5], spectrophotometry [6], fluorimetry [7] and capillary electrophoresis [8]. Although these methods are successfully used to determine Tryp, they have several drawbacks including long analysis time, poor reproducibility, pre-cleaning of the matrix, pre-treatment of the sample and low detection limits. On the other hand, electrochemical method of determination is less expensive, more convenient, ease in handling, high sensitivity and selectivity.

It is well known that higher concentration of ascorbic acid (AA) always co-exists with Tryp in human fluids [9,10]. AA is an effective antioxidant by production of our body against oxidative stress [11]. Further, AA and cupric ions are required for the activation of tryptophan-5-hydroxylase enzyme which involves in the production of 5-hydroxy tryptophan from Tryp [12]. It has been shown that, Tryp recovery has been improved in biological system in the presence of AA [13]. On the other hand, the half-life period of

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paracetamol (PA) in our body can be effectively increased in the presence of AA. PA is commonly used for headaches and minor aches. The high doses of AA lower the amount of PA passed in urine [14]. It will cause the levels of the drugs in our blood to be maintained for long time [15]. Therefore, selective determination of Tryp in the presence of AA and PA and also simultaneous determination of them are very important in clinical analysis.

To the best of our knowledge, only three reports are available in the literature for the simultaneous determination of AA, PA and Tryp [16–18]. Ensafi et al. have reported the determination of AA, PA and Tryp by square wave voltammetry using N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide modified carbon nanotubes paste electrode [16]. The same research group has also used 8,9-dihydroxy-7-methyl-12H-benzothiazolo[2,3-b]quinazolin-12-one-multiwalled carbon nanotubes paste electrode for the determination of these three analytes [17]. Keyvanfard et al. reported the simultaneous determination of AA, PA and Tryp using multi-walled carbon nanotube paste electrode [18]. Even though, these two research groups determined Tryp in the presence of AA and PA, the oxidation potential of Tryp reported in the reported papers appeared at more positive potential (0.85 V) and also the detection limit is only in the range of micro molar concentration. Therefore, it is highly essential to develop a method to determine Tryp at low over potential with high sensitivity. Thus, the aim of the present study is to determine Tryp with high sensitivity and selectivity.

Recently, the electrode modification using multiwalled carbon nanotubes (MWCNTs) has received much attention due to their small size, high specific surface area, good electrical conductivity and strong antifouling property [19,20]. It has been demonstrated that the electrode modified with MWCNTs–polymer composites improved the electrochemical signal in sensors [21]. In the present study, we have attached FMWCNTs on GC electrode using 1,8-octane diamine as a linker with a help of a coupling agent. Then, 5-amino-2-mercapto-1,3,4-thiadiazole is electrochemically deposited on the surface of FMWCNTs to form FMWCNTs/p-AMT composite film. The FMWCNTs and FMWCNTs/p-AMT composites were characterized by SEM and cyclic voltammetry. The SEM images showed the presence of a thin layer of AMT film on the surface of FMWCNTs. The FMWCNTs composite film is used to determine Tryp in the presence of AA and PA. The practical application of the GC/OD/FMWCNTs/p-AMT modified electrode was demonstrated by determining the concentration of Tryp in human blood serum samples.

2. Experimental

2.1. Chemicals

5-Amino-2-mercapto-1,3,4-thiadiazole (AMT), acid functionalized multiwalled carbon nanotubes (FMWCNTs), dicyclohexylcarbodiimide (DCC), 1,8-octane diamine (OD), ascorbic acid (AA), paracetamol (PA) and L-tryptophan (Tryp) were purchased from Aldrich and were used as received. All other chemicals used in this investigation were of analytical grade. Na_2HPO_4 and NaH_2P_4 were used to prepare phosphate buffer (PB) solution (pH 7.2) with double distilled water. GC plates were purchased from Alfa Aesar.

2.2. Instrumentation

Electrochemical measurements were performed in a conventional two-compartment three electrode cell with a mirror polished 3 mm GC electrode as working electrode, Pt wire as counter electrode and NaCl saturated Ag/AgCl as reference electrode. The electrochemical measurements were carried out with a CHI model 634B electrochemical workstation (CH Instruments, Austin, TX, USA). For DPV measurements, a pulse width of 0.06 s, amplitude of 0.05 V, a sample period of 0.02 s and a pulse period of 0.20 s were used. All the electrochemical measurements were carried out under a nitrogen atmosphere at 27 °C.

2.3. Fabrication of FMWCNTs modified GC electrode

The GC electrode was polished with 0.05 μm alumina slurry and rinsed thoroughly with water. Then, the electrode was sonicated in water for 5 min to remove the adsorbed alumina particles. The cleaned GC electrode was immersed in an ethanolic solution 1 mM OD for 8 h. The electrode was washed with ethanol and subsequently with water. The OD modified GC electrode was then immersed into a solution containing (1:1) 0.2 mg/ml FMWCNTs and 2 mM DCC in ethanol for 4 h. The FMWCNTs were attached on the GC electrode through amide bond by the condensation reaction between the amine groups at the terminal end of the SAM and acid groups of the FMWCNTs [22]. For SEM measurements, GC plate was used as a substrate with similar modifications.

2.4. Preparation of CNTs–polymer composite electrode

Electropolymerization of AMT on bare GC and GC/OD/FMWCNTs modified electrodes were carried out by 15 successive potential sweeps between −0.20 V and +1.70 V at a scan rate of 50 mV s^{-1} in 0.1 M H_2SO_4 [23]. After the deposition of the polymer film, the electrode was washed with water and kept in phosphate buffer solution before used for electroanalysis.

3. Results and discussion

3.1. Characterization of MWCNTs modified electrode

The attachment of FMWCNTs on electrode surface was characterized by using CV technique. Fig. 1 shows the cyclic voltammograms (CVs) obtained for bare GC, GC/OD and GC/OD/FMWCNTs modified electrodes in the presence of 3 mM $[Ru(NH_3)_6]^{3+}$ containing 0.2 M PB solution (pH = 3) at a scan rate of 50 mV s^{-1}. At bare GC electrode, it shows a reversible redox peak with a peak separation of 70 mV corresponding to the $[Ru(NH_3)_6]^{3+/2+}$ redox couple (curve a). In the case of GC/OD, 120 mV peak separation with decreased redox current was observed (curve b). The passivation of the $[Ru(NH_3)_6]^{3+/2+}$ electrochemistry at GC/OD modified electrode surface was due to the electrostatic repulsion between the

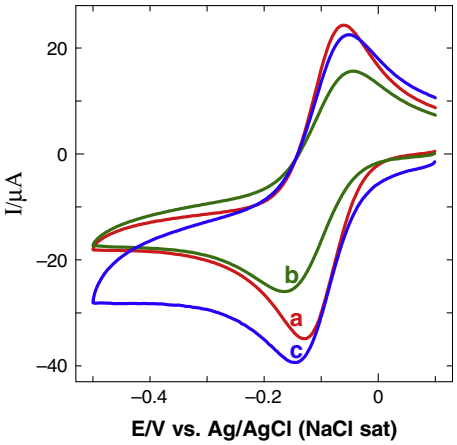

Fig. 1. CVs obtained for (a) bare GC, (b) GC/OD and (c) GC/OD/FMWCNTs modified electrodes in 0.2 M phosphate buffer (pH = 3) containing 3 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 50 mV s^{-1}.