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Multi-walled carbon nanotube modified carbon paste electrode as a sensor for the amperometric detection of L-tryptophan in biological samples

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ABSTRACT

An electrochemical sensor for the amperometric determination of L-tryptophan (Trp) was fabricated by modifying the carbon paste electrode (CPE) with multi-walled carbon nanotubes (MWCNTs) using drop cast method. 4.0 μL of the dispersion containing 2.0 mg of MWCNTs in 1.0 mL of ethanol was drop cast onto the electrode surface and dried in hot air oven to form a stable layer of MWCNTs. The electro-catalytic activity of the modified electrode towards the oxidation of Trp was thoroughly investigated. The modification with MWCNTs has greatly improved the current sensitivity of CPE for the oxidation of Trp. A very minimal amount of the modifier was required to achieve such a high sensitivity. The field emission scanning electron microscopy (FESEM) images revealed a uniform coverage of the surface of CPE by MWCNTs. Nyquist plots revealed the least charge transfer resistance for the modified electrode. The analytical performance of the modified electrode was examined using amperometry under hydro-dynamic conditions. The two linear dynamic ranges observed for Trp were 0.6–9.0 μM and 10.0–100.0 μM. The amperometric determination of Trp did not suffer any interference from other biomolecules. The detection limit of Trp at modified electrode was $(3.30 \pm 0.37) \times 10^{-8}$ M ($S/N = 3$). The analytical applications of the modified electrode were demonstrated by estimating Trp in the spiked milk and biological fluid such as blood serum. The modified electrode showed good reproducibility, long-term stability and anti-fouling effects.

1. Introduction

Tryptophan (Trp) is one of the most important essential amino acids having biochemical, nutritional and clinical significance in humans and herbivores [1]. Trp is commonly synthesized in plants and microorganisms from shikimic acid [2]. Trp is abundantly present in oats, milk, chocolates, bananas, yogurt, dried dates, etc. as component of dietary protein while it is scarcely present in vegetables. Hence, it has been supplied through food products to avoid its deficiency [3–5]. Trp is one of the basic constituents of protein and a requisite in human nutrition to establish and maintain a positive nitrogen balance [6]. Trp acts as precursor for serotonin, melatonin and niacin [7]. Improper metabolism of Trp accumulates toxic products in brain which causes hallucinations, delusions and schizophrenia [8]. An overdose of Trp creates drowsiness, nausea, dizziness and loss of appetite [9]. The level of Trp in blood closely relates to serotonin and melatonin level in the brain while the same in plasma relates to hepatic disease [10]. Hence, Trp is very essential for people with anxiety, sleep deprivation and the need for mood enhancement. It is also used as a sleep aid, nutraceutical and antidepressant [11]. Therefore, developing a simple, fast, inexpensive and accurate method for the determination of Trp in food products, pharmaceuticals and biological fluids is necessary and is likely to have great significance in life science research and drug analysis.

Several methods that have been used for the determination of Trp in different samples include chromatography, chemiluminescence, spectrophotometry, fluorimetry, flow injection analysis and electrophoresis [12,13]. Nevertheless, these methods are complex, time-consuming, expensive and often suffer from selectivity or specificity and pretreatment or require derivatization prior to its determination [14]. Trp being an electroactive compound, electroanalytical techniques provide an alternate way to analyze Trp with certain advantages such as quick response, high sensitivity, high selectivity, inexpensiveness, amenability to miniaturization, low power consumption and wide linear dynamic range [15]. However, the electrochemical detection of Trp faces some problems. At traditional working electrodes, Trp follows a sluggish kinetics and

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has very high oxidation overpotential [16,17]. The other electroactive biomolecules which coexist with Trp in biological matrices interfere with the determination of Trp due to their similar oxidation peak potentials. These problems are solved by modifying the electrodes with suitable materials using various modification methods [18]. Many materials have been used to modify the traditional working electrodes for the determination of Trp [19]. The carbon paste electrode (CPE) has been used in the development of electrochemical sensors for various biologically significant molecules because of its simple method of preparation, renewable surface, low background current, applicability of various modification procedures and above all, its biocompatibility [20–22].

Carbon nanotubes (CNTs) are most promising materials to modify the electrodes because of their unique ability to promote electron transfer kinetics [23]. CNTs are of ultra-light weight with excellent electronic conductivity, high surface area and have thermal and chemical stability [24]. Basically, there are two types of CNTs – single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWCNTs contain single graphene roll while MWCNTs contain many such concentric graphene rolls [25]. Different types of CNT modified electrodes are reported in literature to study biologically important compounds [26,27]. They are CNT-polymer nanocomposite electrodes, CNT-paste electrodes, CNT/ sol–gel nanocomposite electrodes and layer-by-layer assembly of CNT film electrode. MWCNTs modified electrodes have also been used for the determination of Trp [28].

Casting is a common route to modify the electrodes. To date, extensively used surfaces for casting are glassy carbon, gold and graphite electrodes, but the surface of carbon paste is not commonly used for this kind of modification. Unlike the other methods of modification, in casting, the modifier covers the entire surface of the electrode and is completely exposed to analyte solution. Hence, it can improve the analytical performance of the electrode in terms of selectivity or specificity towards a particular analyte. A minimum amount of the modifier is used in this kind of modification. Casting of MWCNTs films at CPE have not yet been reported for the determination of Trp.

In continuation of our efforts to use CPE and modified CPE for different applications [29–33], our objective in the present work was to develop a simple amperometric sensor for the determination of Trp which is free from all interfering molecules. Even though there are several reports for the fabrication of a sensor for the determination of Trp, the focus happens to be on complicated procedures of modifications. Hence, a sensor with a simple method of preparation is much desired. In this study, our aim was to use a simple and convenient method of modification i.e. casting the MWCNTs on a carbon paste as the underlying surface in order to fabricate a highly sensitive sensor with better lower detection limits and free from interferences from other bio-molecules which coexist with Trp. Electrochemical investigation of Trp at this modified electrode reveals its electro-catalytic activity towards Trp. Analytical applications of the modified electrode were demonstrated by estimating the Trp content in milk and biological sample such as the blood serum.

2. Experimental

2.1. Reagents

L-Tryptophan, L-Tyrosine, L-Cysteine, Uric acid, Folic acid (SRL), Dopamine hydrochloride, Epinephrine hydrochloride (Aldrich), Acetaminophen (Micro Labs Ltd.), Ascorbic acid, KH_2PO_4, H_3PO_4, NaOH pellets and $HClO_4$ (Merck) were of analytical grade and used as received. All aqueous solutions were prepared with ultra pure water (>18.2 MΩ cm) from Milli-Q Plus system (Millipore). Stock solutions of EP, DA were prepared in 0.1 M $HClO_4$, UA, L-Cysteine, Folic acid and L-Tyrosine in 0.1 M NaOH and L-Tryptophan, Acetaminophen and Ascorbic acid in water. Phosphate buffer solutions were prepared from KH_2PO_4 and NaOH and pH was adjusted using H_3PO_4 or NaOH. The graphite powder was obtained from Graphite India Ltd.. The thin MWCNTs obtained from Nanocyl SA (Belgium) were synthesized by decomposition of ethylene using the combustion chemical vapor deposition method. The MWCNTs have an average diameter of 10.0 nm and length of several (0.1–10.0) μm.

2.2. Apparatus

All electrochemical experiments were performed using Chemi-Link model EA-201 Electro Analyzer. A conventional three electrode system was used for all electrochemical experiments, which comprise a bare or modified CPE as working electrode, a platinum wire as auxiliary electrode and all potentials were measured and applied using saturated calomel electrode (SCE) as a reference electrode. The tip of Lugin capillary was set approximately at a distance of 1 mm from the surface of the working electrode – bare and modified CPE in order to minimize the error due to IR drop in the electrolyte. The cyclic voltammetric (CV) studies were performed in quiescent solution and the amperometric experiments were carried out under the hydrodynamic conditions. The electrochemical experiments and voltammetric curves were recorded at room temperature (~300.0 K). The surface morphology of the electrodes was studied using field-emission scanning electron microscopy (FESEM) using Quanta 200, FEI, Germany; SUPRA 40 VP, Gemini, Zeiss, Germany. Electrochemical impedance spectroscopy (EIS) was performed using VersaSTAT 3. A digital pH/mV meter (ELICO LI 614) was employed to measure the pH of the prepared buffer solutions.

2.3. Generation of oxygen functionalities on MWCNTs

The oxygen functionalities on the surface of MWCNT are known to improve their electrochemical properties. Hence, the same were generated by treating them with a mixture of concentrated H_2SO_4 and HNO_3 (molar ratio 3:1). In a typical experiment 75.0 mL of conc. H_2SO_4 (97%) and 25.0 mL of conc. HNO_3 (65%) were mixed and added to 1.0 g of MWCNTs in a round-bottomed flask and heated under constant agitation at 50 °C for 8.0 h. It was allowed to cool down to room temperature after which an equal quantity of deionised water was added. It was filtered and the residue was washed several times with deionised water until neutral pH was attained. The residue was then filtered and freeze-dried [34].

2.4. Preparation of bare and MWCNTs modified carbon paste electrodes

After optimization of the ratio of graphite powder to binder, the CPE was prepared by thoroughly hand-mixing the graphite powder and silicone oil in the ratio 70:30 (w/w) in an agate mortar using a pestle to obtain a homogeneous paste. A portion of the resulting homogeneous paste was packed into the cave of the Teflon tube. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical contact with the external circuit.

2.0 mg of MWCNTs were dispersed in 1.0 mL of ethanol under ultrasonication to prepare MWCNTs modified carbon paste electrode. 4.0 μL of the above dispersion was drop cast onto the electrode surface and dried in hot air oven to form a stable layer of MWCNTs. The prepared electrode is designated as MCPE/MWCNTs. The surface morphology of CPE and MCPE/MWCNTs was analyzed by recording the FESEM images as shown in Fig. 1a and b.