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Electropolymerization of bromothymol blue on carbon paste electrode bulk modified with oxidized multiwall carbon nanotubes and its application in amperometric sensing of epinephrine in pharmaceutical and biological samples

Prerna Pradhan [a], Ronald J. Mascarenhas [b,*], Tony Thomas [b], Irishi N.N. Namboothiri [c], Ozma J. D'Souza [b], Zineb Mekhalif [d]

[a] Department of Postgraduate Studies and Research in Chemistry, St. Joseph's College (Autonomous), Lalbagh Road, Bangalore 560027, Karnataka, India
[b] Electrochemistry Research Group, Department of Chemistry, St. Joseph's College (Autonomous), Lalbagh Road, Bangalore 560 027, Karnataka, India
[c] Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400 076, India
[d] Laboratoire de Chimie et d'Electrochimie des Surface, University of Namur, 61 Rue de Bruxelles, B-5000 Namur, Belgium

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ABSTRACT

A composite electrode for an amperometric detection of epinephrine (EP) has been developed by electropolymerizing bromothymol blue (BTB) on carbon paste electrode (CPE) bulk modified with multi-walled carbon nanotubes (MWCNTs). The field-emission scanning electron microscopy and the energy dispersive X-ray analysis (EDX) confirm the formation of poly(bromothymol blue). A possible mechanism of electropolymerization of BTB has been proposed. The electropolymerization of BTB on the surface of CPE involves much less energy as compared to that of glassy carbon surface. The modification enhanced the current sensitivity of EP by 5.5 times as compared to the bare CPE. The electrochemical impedance spectroscopy (EIS) studies revealed a least charge transfer resistance (R_{ct}) for the modified electrode. The sensor showed an optimum current response at physiological pH and the response was linear for the concentration of EP in the ranges 0.8–9.0 μM and 10.0–100 μM, with a detection limit of 8×10^{-7} M. The amperometric response of EP remains unaltered even in the presence of 50-fold excess of uric acid, ascorbic acid and 100-fold excess of L-Tryptophan, L-Tyrosine, L-Cysteine and Nicotinamide adenine dinucleotide. This sensor has shown stability, reproducibility, anti fouling effects and was successfully applied for the determination of EP in blood serum and adrenaline injection.

1. Introduction

Epinephrine (EP), otherwise known as adrenaline is a hormone secreted by the medulla of the adrenal glands. It belongs to the family of catecholamine and plays an important function as a neurotransmitter in the mammalian organisms [1,2]. This hormone is released into the bloodstream as a response to fear, anger or excitement, which results in an increase in the blood sugar level. Hence, it is also called as flight or fight hormone [3]. Abnormalities of EP levels in the body give rise to several disorders such as Parkinson's disease [4]. Clinically appropriate dosage of EP has been used as a common emergency health care medicine to treat cardiac arrests, cardiac dysrhythmia and as a bronchodilator for asthma [5,6]. The quantitative determinations of EP have gained a lot of importance in clinical diagnosis and related pharmacological research. There are various methods for detection of EP such as liquid chromatography [7], chemiluminescence [8], flow injection method [9] and capillary electrophoresis method [10]. The electroanalytical methods are efficient means for the detection of catecholamines [11,12]. The electroanalytical methods are superior to all the above mentioned methods since they are simple, fast, require a small amount of the sample for detection and are inexpensive [13,14]. One of the challenges faced in the electrochemical detection of EP is the interference from other molecules such as Uric Acid (UA), Dopamine (DA) and Ascorbic Acid (AA). Another factor which limits the detection of EP at various electrodes is large overpotential due to its irreversible electrochemical nature [15–17]. However, the various ways of modification of the working electrode can overcome these problems which can result in increased sensitivity and efficiency of detection.

* Corresponding author. Tel.: +91 9448756584; fax: +91 8022245831.
E-mail address: ronaldmasc2311@yahoo.co.in (R.J. Mascarenhas).

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CPE is extensively used as an electrode material especially for sensing the bio molecules on account of its biocompatibility, inexpensiveness and electrochemical inertness in the potential window of interest. They are easy to prepare and handle, and they can be manipulated with various modifiers and mediators in order to improve the kinetics of numerous electroactive biomolecules [18,19]. Modification of the CPE is done by introducing an electrochemically active surface onto the material [20,21].

Carbon nanotubes (CNTs) have achieved a remarkable utility in the field of electrochemistry [22]. CNTs have proved to be promising as modifiers in the field of electrochemical sensors over the past few years [23]. The physical and catalytic properties of CNTs make them ideal for their use in sensing applications. The electrodes modified by CNTs usually have improved electron transfer kinetics, sensitivity and lower detection limits [24]. Depending on the necessity, the single-walled carbon nanotubes (SWNTs), multi-walled carbon nanotubes (MWCNTs) and various functionalized CNTs have been used as modifiers for the detection of catacholamines [25,26].

The modification of electrodes by electropolymerization has been extensively employed for the detection of analytes as it has been proved to be advantageous in many ways. Polymer modified electrodes show high catalytic ability, absence of surface fouling and it eliminates unwanted reactions competing with the kinetics of desired electrode processes [27,28]. The enhanced electro-catalytic ability can be explained in terms of increase in peak current and lowering of overpotential [29]. The electrochemical behaviour of EP has been studied at electrodes polymerized with different molecules such as poly(methylene blue) [30], ploy(eriochrome black T) [31], and poly(p-xylenolsulfonephthalein) [32]. To the best of our knowledge, we have not come across any report in the literature wherein poly(bromothymol blue) is being used for the detection of EP or of CPE being used as underlying surface for electropolymerization.

Bromothymol blue (BTB) is a chemical indicator for weak acids and bases. It is a probe highly sensitive to the alteration of structural as well as physical properties of proteins, bio membranes, and phospholipid vesicles. The electrochemical behaviour of BTB has been studied previously using cyclic voltammetry (CV) [33].

In our effort to modify the CPE and also use them for different applications, [34–38], in the present study, we aim to fabricate a modified CPE which is free from the interference of co-existing molecules such as UA, DA and AA and to authenticate its applicability as a sensor for the determination of EP in pharmaceuticals as well as in biological samples. For this purpose we carried out electropolymerization in order to immobilize the polymer on the surface of CPE. Our study suggests CPE as a better surface to carry out electropolymerization as compared to the surface of glassy carbon electrode (GCE). Further, we wanted to investigate the influence of MWCNTs on the surface of CPE and therefore, we carried out modification of CPE by oxidized MWCNTs ($MWCNT_{ox}$) in two ways; by bulk modification and by drop cast method. The electropolymerization was carried out over the surface of these modified electrodes and the behaviour of EP was studied using CV technique. Also, we observed that this particular type of modification had enhanced the oxidation peak current of EP to a greater extent as compared to our previously reported work [39]. This fact led us to further investigate the possibility of using this composite electrode material for the trace level detection of EP. The results were better at BTB electropolymerized surface of CPE bulk modified with $MWCNT_{ox}$ than many other modified electrodes as discussed quantitatively in Table 1. The analytical characterization and analytical applications of the modified electrode for the estimation of EP was achieved using amperometry. This modified electrode exhibited good stability, reproducibility, enhanced selectivity and sensitivity for the oxidation of EP even in the presence of other interfering molecules.

2. Experiment

2.1. Reagents and solutions

Epinephrine hydrochloride, Isoproterenol hydrochloride, Levodopa and Methyldopa (Aldrich), Noradrenaline bitartrate (NORAD®, NEON Laboratories Limited, India), L-Tryptophan (Trp), L-Tyrosine (Tyr), L-Cysteine (Cys), UA (SRL), Nicotinamide adenine dinucleotide (NADH) (Spectrochem), AA, KH_2PO_4, H_3PO_4, NaOH pellets, $HClO_4$, silicone oil (Merck) and bromothymol blue (Thomas Baker) were of analytical grade and used as received. Graphite powder was obtained from Graphite India Ltd. All aqueous solutions were prepared with ultra pure water (>18.2 MΩ cm) from Milli-Q Plus system (Millipore). Stock solutions of EP, UA and AA (25.0×10^{-3} M) were prepared in 0.1 M $HClO_4$, 0.1 M NaOH and water respectively. Phosphate buffer solutions (PBS) were prepared from KH_2PO_4 and NaOH and the pH was adjusted using H_3PO_4 or NaOH. The MWCNTs obtained from Nanocyl SA (Belgium) were synthesized by decomposition of ethylene using the combustion chemical vapour deposition method. The MWCNTs have an average diameter of 10 nm and length of several (0.1–10) μm.

2.2. Apparatus

All CV and amperometry studies were done using three-electrode system consisting of either CPE or modified CPE as the working electrode. Platinum electrode was used as an auxiliary electrode and saturated calomel electrode (SCE) was used as the reference electrode. The tip of the Lugin was kept at a fixed distance from the working electrode surface throughout the experimental studies. All experiments were carried out at room temperature using ChemiLink EA-201 Electro Aanlyzer controlled by a personal computer. The surface morphology of the electrodes were studied using field-emission scanning electron microscopy (FE-SEM) and EDS using Quanta 200, FEI, Germany; SUPRA 40 VP, Gemini, Zeiss, Germany. The pH values of PBS were measured using a digital pH/mV meter (ELICOLI641). Electrochemical impedance spectroscopy (EIS) was performed using VersaSTAT 3.

2.3. Generation of oxygen functionalities on MWCNTs

Since the oxygen functionalities on the surface of MWCNTs improve their electrochemical properties, the oxygen functionalities were generated by treating them with a mixture of concentrated H_2SO_4 and HNO_3 (molar ratio 3:1). For this purpose, a 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 50.0 °C for 8.0 h. It was allowed to cool down to room temperature after which an equal quantity of deionized water was added. It was filtered and the residue was washed several times with deionized water until neutral pH was attained. The residue was then filtered and freeze-dried [40]. The oxidized MWCNTs are abbreviated as $MWCNTs_{ox}$.

2.4. Preparation of carbon paste electrode and MWCNTs modified carbon paste electrode

The ratio of graphite powder to binder was optimized for the best result. The bare CPE was prepared by thoroughly mixing the graphite powder with silicone oil in the ratio 70:30 (w/w) in an agate mortar and pestle till a homogeneous paste was obtained. This mixture of bare carbon paste was filled into the Teflon electrode cavity and was tightly packed and then it was smoothened to obtain a uniform surface.