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Graphene modified Palladium sensor for electrochemical analysis of norepinephrine in pharmaceuticals and biological fluids

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ABSTRACT

A novel voltammetric sensor for the determination of norepinephrine (NE); an important neurotransmitter, has been developed using graphene modified palladium (Pd) sensor. Graphene was synthesized in the laboratory and was characterized by SEM, TEM, XRD and Raman spectroscopic measurements. The electrochemical response of modified sensor towards NE determination was studied by the means of electrochemical impedance spectroscopy, cyclic voltammetry and square wave voltammetry. The modified sensor exhibited excellent electrocatalytic activity for the oxidation of NE, leading to a remarkable enhancement in the peak current and lowering of peak potential. The anodic peak current was found to increase linearly with increasing the concentration of NE using graphene modified Pd sensor in the range 0.0005–0.5 mM in phosphate buffer of pH 7.2 with limit of detection and limit of quantification as 67.44 nM and 224.8 nM, respectively. The detection sensitivities of bare and modified Pd sensors were 0.851 $\mu A\,mM^{-1}$ and 17.428 $\mu A\,mM^{-1}$, respectively, revealing the good electrocatalytic properties of graphene modified sensor. The proposed method was successfully applied for the determination of NE in pharmaceutical dosage forms and human urine samples. It was observed that using developed sensor NE can be detected in urine samples even in the presence of high concentration of uric acid.

1. Introduction

Graphene, one of the important carbon nano materials has attracted enormous attention of scientific world in recent years because of its excellent properties such as high electrical and thermal conductivity, good chemical stability, great mechanical strength, large surface to volume ratio and broad electrochemical window [1–6]. Graphene is a two dimensional carbon nano material with sp^2-hybridized carbon atoms arranged in six-membered rings. A weak π bond is formed using half filled $2p_z$ orbital, resulting in delocalized π electron clouds. The unique electronic and transport properties of graphene are attributed to these delocalized π electron clouds [7,8]. Several methods including mechanical exfoliation of highly ordered pyrolytic graphite [9,10], epitaxial growth [11,12] and chemical vapor deposition [13,14] are in use for the synthesis of graphene, however for the electronalytical purposes, graphene is synthesized by the reduction of graphite oxide (GO). Graphene synthesized from GO reduction has abundant structural defects and functional groups and is found to retain the conjugated network of graphitic lattice [15–17]. It is observed that graphene-based nanomaterials have a large accumulation capability due to their high surface area [6], hence, are considered as ubiquitous substrate for trace accumulation of analytes especially aromatic molecules, which are expected to adhere with the sp^2 structure of graphene sheets via π–π stacking interactions [18].

The last decade has seen the applications of Pd as electrode material and as nanoparticle for modification of the electrode surface in the field of analysis of biomolecules and drugs due to its unique mechanical and electrical properties [19–22]. Pd exhibits chemical inertness and is conductive in nature. It is biocompatible and shows strong affinity towards the organic molecules. It provides low background current and shows excellent stability and reproducibility during the experiments [23–27]. Palladium is best suited for the graphene modification as it has tendency to get chemisorbed on to palladium surface, which is not the case with diamond and carbon fiber electrodes. In addition Palladium is conducting and is quite strong and stable in comparison to the diamond electrode, which itself is insulating and requires doping with boron or nitrogen in order to be used as electrode for electrochemical studies. Carbon fiber electrode is found to be delicate and thus its practical handling is quite difficult. Moreover, graphene chemisorbs over palladium surface through strong interactions and

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get deposited to form stable modification [28]. Hence, Pd is aptly chosen as the substrate for graphene modification in these studies.

Norepinephrine (NE, I) or noradrenaline is a catecholamine, which acts as hormone and neurotransmitter. The human adrenal medulla releases about 20% NE, while the adrenergic neurons are responsible for the major NE production [29]. The biosynthesis of NE in human system begins with tyrosine which is produced in the liver from phenylalanine [30]. Tyrosine is then transported to catecholamine secreting neurons and adrenal medullary cells, where it gets converted into dopamine (DA) through a series of reactions. In neurons, DA is converted to NE in presence of an additional enzyme *dopamine-β-hydroxylase* [30]. NE promotes the conversion of glycogen to glucose in the liver and helps in converting the fats into fatty acids, resulting in an increment in energy production [31]. NE is also responsible for the increased heart rate and blood pressure, dilation of pupils, dilation of air passages in lungs and narrowing of blood vessels due to which body is enabled to perform well in stressful situations. It is also used as performance enhancing drug in competitive games by athletes; therefore, prohibited by the World Anti-Doping Agency [32]. The quantification of NE concentration in biological system provides important information about its physiological adverse effects such as anxiety, diabetes, pain, heart disease and other neurological disorders like Parkinson and Alzheimer diseases [33–37]. Therefore, it is essential to establish a rapid, reliable and sensitive method for the direct determination of NE in pharmacological formulations as well as in biological samples.

Several analytical methods such as capillary electrophoresis [38], spectrophotometry [39] and high-performance liquid chromatography [40] have been executed for the determination of NE. These methods are rather complicated and expensive, moreover long time is required for derivatization, extraction and purification of the species prior to their determination using these methods. The utilization of electrochemical techniques in analytical chemistry has gathered much attention of researchers owing to their rapidity of analysis, no requirement of sample pretreatment, relatively high sensitivity and inexpensive instrumentation.

In this paper we describe the application of graphene modified palladium (Pd) sensor for the electrochemical analysis of NE for the first time. Although several methods are described in literature for the determination of NE in biological samples, still it is a challenging task to detect its low concentration and to develop a sensor free from surface fouling effect. By using graphene modified Pd sensor we have successfully determined its concentration upto nM detection limit, which is quite comparable with previously reported methods [41,42]. Furthermore, the developed sensor solved the problem arising from the presence of common metabolites of urine such as uric acid (UA) and ascorbic acid (AA), which get oxidize at the oxidation potential close to NE and interfere in its determination in urine samples. The graphene modified sensor not only exhibited excellent electrocatlytic activity towards the NE detection but also resolved the overlapping peaks of NE and urine metabolites into well-defined separate peaks. The voltammetric response of NE was not affected in the presence of high concentration of uric acid and ascorbic acid. The modified sensor shows high sensitivity, good stability, reproducibility and is practically very useful for the electrochemical sensing of NE in pharmaceuticals and physiological fluids.

2. Structure I here

2.1. Experimental

2.1.1. Reagents and Instrumentation

NE, graphite powder (< 20 μm), hydrazine and sulphuric acid were obtained from Sigma Aldrich, USA. Phosphate buffers of different pH (μ = 1 mol L^{-1}) were prepared using the method of Christian and Purdy [43]. Noradrenaline bitartarate injection (Neon Labs. Ltd.; Mumbai, Maharashtra, India) was obtained from Institute hospital of Indian Institute of Technology Roorkee. All other reagents and solvents used during the experiment were of analytical grade.

All voltammetric experiments were performed using Bioanalytical system (BAS, West Lafayette, USA) Epsilon EC-USB voltammetric analyzer equipped with three electrodes in a single compartment glass cell. An Ag/AgCl (3 M NaCl) was used as reference electrode (BAS Model MF-2052 RB-5B), Pt wire as counter electrode and Pd electrode as working electrode. The pH of buffer solutions was measured by using digital pH meter (Eutech Instruments, model pH 700).

Powder X-ray diffraction (XRD) measurements were performed using Bruker D8-advance X-ray powder diffractometer. The Transmission electron microscopic measurements (TEM) were carried out using TEM model; Technai G^2 20S-TWIN. Raman spectroscopic measurements were performed on Renishaw invia Raman microscopy. The characterization of electrode surface was carried out using Field emission scanning electron microscopy (FE-SEM, model; Zeiss ultra plus 55). Electrochemical impedance spectroscopy (EIS) was performed on galvanostat (model; Versastat 3, PAR).

2.2. Synthesis of Graphene

The graphene was synthesized using the improved Hummers method [44,45]. This method is different from other methods [46] in respect that ammonia had not been used while reducing GO with hydrazine. It was synthesized in two steps. In first step graphite oxide (GO) was synthesized using graphite powder. In the second step GO thus obtained was chemically reduced to graphene. For the synthesis of GO, 9 g of $KMnO_4$ was added to 1.5 g of graphite powder and the reaction was carried out in presence of H_3PO_4 and H_2SO_4 (20: 180) mixture. The mixture was then heated to 50 °C and stirred for 12 h, after which a dark brown colored material was obtained. Then, addition of 100 mL of water, followed by the slow addition of 1.5 mL of H_2O_2 (30%), changed the color of the solution to yellow. The solution was then centrifuged and the sediment thus obtained was washed with 200 mL of water followed by washing with 100 mL of HCl (30%). Further washing was done with 200 mL of ethanol and final sediment so obtained was GO.

For the synthesis of graphene, reduction of GO was carried out by adding 20 mL of water to 50 mg of GO powder followed by the addition of 0.5 mL of hydrazine. Contents were then sonicated for 1 h followed by stirring for 24 h at 50 °C. After filtration black powder of graphene was obtained, which was dried in vacuum. GO and graphene as obtained were characterized using XRD, TEM and Raman spectroscopic measurements.

2.3. Preparation of graphene modified electrode

For the fabrication of graphene on Pd electrode, the surface of Pd was first polished using alumina (grade I) and zinc oxide on microcloth pad. Suspension of graphene was prepared by dispersing 7 mg of graphene in a mixture of double distilled water and DMF (1: 9) according to the previously reported method [47] at a concentration of 0.7 mg mL^{-1}. The amount of graphene casted on the electrode surface was optimized by casting its different amount in the range 2-20 μL (Fig. 1) and then electrochemical response of NE (0.2 mM) was checked. The maximum peak current was observed when we used 7 μL (equivalent to 60 μg per unit area of electrode), therefore it was selected as an optimum amount. This optimum amount of graphene was casted on the electrode surface of Pd and