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Graphene modified glassy carbon sensor for the determination of aspirin metabolites in human biological samples

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ABSTRACT

A graphene modified glassy carbon (GR/GCE) sensor has been developed for the determination of aspirin metabolites 2,3- and 2,5-dihydroxybenzoic acids (2,3- and 2,5-DHB). The modified sensor was characterized by Field Emission Scanning Electron Microscopy and Electrochemical Impedance Spectroscopy. The electrochemical behavior of 2,3- and 2,5-DHB was investigated by cyclic and square wave voltammetry. The modified sensor exhibited excellent electrocatalytic activity for the oxidation of 2,3- and 2,5-DHB, leading to a remarkable enhancement in the peak current as compared to the bare sensor. The results were attributed to the enhanced surface area and high conductivity of GR. The anodic peak currents of 2,3- and 2,5-DHB were found to be linear in the concentration range of 1–150 μM and 1–200 μM with the detection limits of 47 nM and 51 nM, respectively. The sensor was capable to determine 2,5-DHB effectively without any interference from the uric acid and other metabolites present in the urine samples. The practical utility of GR/GCE has been successfully demonstrated for the determination of 2,5-DHB in the urine samples of persons undergoing treatment with aspirin.

1. Introduction

Aspirin, also known as acetylsalicylic acid (ASA) is often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication [1–3]. ASA is also used in cardiovascular diseases, as it prevents heart attacks, strokes and blood clot formation in high risk patients [4,5]. 2,3-Dihydroxybenzoic acid (2,3-DHB) and 2,5-dihydroxybenzoic acid (2,5-DHB) are the active biological oxidative derivatives of salicylic acid (SA), out of which 2,5-DHB has been found as the major metabolite of ASA. The minor metabolite, 2,3-DHB is a potentially useful iron-chelating drug having antimicrobial properties and has been reported to decrease acute lung injury mediated by neutrophil activation and consequent free radical production. 2,5-DHB came into the prominence as a result of the finding that it possessed a therapeutically useful antirheumatic and antioxidant activity. 2,5-DHB inhibits the polymorphonuclear blood leukocytes aggregation and hyaluronidase in human blood effectively, while SA and ASA are significantly less effective. It has been reported that in the case of high fever conditions, the excretion of 2,5-DHB is remarkably increased in urine as compared to SA [6–11]. About 60% of SA remains unmetabolized in the human systems and reacts with OH free radical to produce 2,3-DHB and 2,5-DHB. Thus, both metabolites are considered as useful marker of in-vivo OH radical production [7,12]. Both the metabolites of aspirin exhibit a broad spectrum of biological activity, hence, it is considered desirable to determine them in biological samples. The determination of aspirin has extensively been carried out and also recently been reported [13], however, very a few attempts have been made to determine metabolites 2,3- and 2,5-DHB of aspirin and techniques such as high performance liquid chromatography (HPLC) coupled with UV or electrochemical determination, gas chromatography–mass spectrometry and spectrofluorimetry are used [14–18]. However, these techniques require heavy and expensive instruments with sophisticated processes, tedious time consuming pretreatment, derivatization and utilize organic solvents for separation. Electrochemical techniques on the other hand are considered ecofriendly and have high selectivity, sensitivity, reproducibility, rapid response and are low cost [19,20].

Graphene (GR) is a monoatom thick planar sheet of sp^2 bonded carbon atoms in a honeycomb crystal lattice. In more complex terms, GR is the basic structure of all graphitic materials and is an allotrope of carbon in the structure of a plane of sp^2 bonded carbon atoms

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arranged in six membered rings through weak π–π interactions. Recently, GR has been claimed as the rising star material because of its novel properties, such as it is thinnest material, flexible, harder than diamond and conducts electricity at room temperature efficiently [21–25]. GR has enticed great attention in scientific research world and is contributing in the field of electrochemical and biosensing, energy storage, solar power, and thermoelectric energy. Molecular sensing can be achieved by the use of GR, since electronically it is a good low-noise material and has been extensively used for the surface modification in electrochemistry. The high electrical conductivity, high thermal conductivity, good chemical stability, large surface area and low cost have made it extremely useful material in electroanalysis. In comparison with carbon nanotubes (CNT), GR has an advantage like very large surface area, large electrical conductivity and does not possess embedded metallic impurities. Moreover, production of graphene uses graphite, which is cheap and easily accessible [26–29]. Among the reported methods, chemical reduction of graphite oxide (GO) is economical and has been widely used [30–32].

In this study, graphene modified glassy carbon electrode (GR/GCE) has been used for the determination of metabolites of aspirin, 2,3- and 2,5-DHB in the presence of salicylic acid. Several analytical methods have been employed to detect these aspirin metabolites, but to the best of our knowledge no voltammetric method has been reported to detect 2,3- and 2,5-DHB in the human biological fluids so far. The purpose of selecting GR in the present studies is absence of embedded metallic impurities. Such metallic impurities present in CNTs strongly affect the peak potential and peak current of analytes [33]. In addition, getting highly pure CNTs is difficult. In our studies GR/GCE showed significant enhancement in oxidation peak current of 2,3- and 2,5-DHB in comparison to gold nano-particles and CNTs and was able to resolve the overlapping peaks of 2,5-DHB and UA into well-defined separate peaks. Hence, the present studies were carried out using GR modified GCE the analytical applicability of the proposed method successfully demonstrated by the graphene modified GCE.

2. Experimental

2.1. Instrumentation

All voltammetric experiments were carried out using Bioanalytical system (BAS, West Lafayette, USA) Epsilon EC-USB voltammetric analyzer equipped with a single compartment glass cell. The electrochemical cell setup used included glassy carbon electrode (GCE, 3 mm dia.) or modified GCE as a working electrode, an Ag/AgCl (3 M NaCl) (BAS Model MF-2052 RB-5B) as reference and Pt wire as counter electrodes respectively. The pH measurement of the buffer solutions was performed using Thermo Fisher Scientific, Singapore Digital pH meter (Eutech Instruments, model pH 700). Field Emission Scanning Electron Microscopy (FE-SEM, model; Zeiss ultra plus 55) was used to characterize the surface morphology of the GR/GCE. Electrochemical Impedance Spectroscopic (EIS) studies were performed using a galvanostat (model; Versastat 3, PAR).

2.2. Chemicals and reagents

2,3-DHB, 2,5-DHB, graphite powder ($<20\,\mu m$), hydrazine, sulfuric acid, uric acid and salicylic acid were obtained from Sigma Aldrich, USA. Phosphate buffers of different pH ($\mu=1.0$ M) were prepared according to the reported method [34]. The urine samples of patients undergoing treatment with aspirin were obtained from the Institute Hospital of IIT Roorkee, after the permission of the human ethical clearance committee of IIT Roorkee. The anthropometric data of the patients was (1) male, 25 years, (2) male, 22 years and (3) female, 25 years. The urine samples were obtained after 2.5 h of oral administration of aspirin. The samples were suitably diluted to minimize matrix complexity. The solution to be analyzed was transferred into the voltammetric cell without any further pretreatment. The standard addition method was used for the determination of 2,5-DHB in real samples. All other reagents and solvents used during the experiment were of analytical grade and double distilled water was used throughout the investigation.

2.3. Voltammetric procedure

Stock solutions of 2,3- and 2,5-DHB (1 mM) were prepared in double distilled water. The required volume of the stock solution was added to the electrolytic cell containing 2 mL of phosphate buffer solution and final volume was made 4 mL with double distilled water. For square wave voltammetry (SWV) the optimum parameters used were initial (E): 0 mV, final (E): 1000 mV, square wave amplitude (Esw): 25 mV, potential step (E): 4 mV, and square wave frequency (f): 15 Hz. All the potentials are reported with respect to Ag/AgCl reference electrode at an ambient temperature of 25 ± 2 °C.

3. Result and discussion

3.1. Fabrication and characterization of modified graphene sensor

GR was synthesized from the graphite utilizing the improved Hummers method and reduced using hydrazine [35–37]. The characterization of GR was carried out by using powder XRD and Raman spectroscopic measurements. Prior to modification, the surface of glassy carbon electrode (GCE) was first polished using alumina powder (grade I) and zinc oxide on micro-cloth pad to a mirror like finish surface and then it was rinsed with distilled water. The suspension of GR was prepared by dispersing 0.7 mg mL^{-1} of GR in a mixture of double distilled water and DMF (1:9) [37,38]. The amount of GR casted on the electrode surface was optimized by casting its different amounts in the range 2–20 μL and then electrochemical response of 2,3- and 2,5-DHB (50 μM) was recorded. For both the compounds, the maximum peak current was observed at 12 μL, therefore, it was selected as an optimum amount for the subsequent studies. After casting the optimum amount of GR onto the GCE surface, the sensor was kept for drying to overnight at room temperature. The surface morphology of the bare GCE and GR/GCE was studied using FE-SEM. A comparison of the microscopic images observed is shown in Fig. 1 and it can be observed that the part of GR is agglomerated during the preparation of suspension in DMF/H_2O (Fig. 1B).

EIS is a useful technique to study the interfacial properties of surface-modified electrodes. The EIS analysis of GCE and GR/GCE was carried out in 1:1 solution of 5 mM of $K_3[Fe(CN)_6]$ and 0.1 M KCl over the frequency range from 100 kHz to 10 mHz at a potential of 0.05 V. The results were found to fit best to a simple Randles equivalence circuit. The Randles circuit consists of the ohmic resistance (R_s) of the electrolyte solution, the double layer capacitance (C_{dl}), electron transfer resistance (R_{ct}) and the Warburg impedance (Z_W). The parallel combination of R_{ct} and C_{dl} gave rise a semicircle portion obtained at higher frequency and the diameter of semicircle was equal to R_{ct}. The linear part in the Nyquist plot at low frequencies is due to the diffusion process and represents Warburg impedance (Z_W). The resultant Nyquist plots observed for bare GCE and GR/GCE are shown in Fig. 2. At unmodified GCE, semicircle with the large diameter was obtained and a charge transfer resistance for the $Fe[CN]_6^{3-/4-}$ redox