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Selective electrocatalysis of reduced graphene oxide towards hydrogen peroxide aiming oxidases-based biosensing: Caution while interpreting

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ABSTRACT

Graphene is currently often reported as a superior electrochemical (and not only) material. Here we assess critically the often-reported electrocatalytic behaviour of an electrode modified with reduced graphene oxide (rGO) towards H_2O_2. Two different electrode materials – gold and glassy carbon – were modified with rGO and comparative measurements in the presence of H_2O_2 and two ordinary electrochemical interferents – ascorbic acid and dopamine – were performed. Neither selectivity nor significant electrocatalytic abilities of rGO modified electrodes were observed. Only the mass transfer was enhanced due to the improvement of the parameters applicable for general electrochemistry on nanoparticles – diffusion layer and electroactive area.

1. Introduction

Graphene – a two-dimensional (2D) single-layered carbon atoms with an sp^2 configuration in a honeycomb (hexagonal) orientation – attracted wide attention in recent years. Together with its derivatives, such as graphene oxide, graphene nanoribbon, chemically or electrochemically reduced graphene oxide has shown some interesting properties such as large surface area ($\sim$2630 $m^2 g^{-1}$ for single-layered material), unique thermal and electric conductivity and excellent mechanical properties [1–5].

In the last few years, such graphene variants showed wide applicability in different fields but most importantly in bioelectronics and biosensing [4,6–10]. According to the literature, graphene appears to be an ideal material for electrochemistry applications [4,6] because of its large surface area, low production costs and excellent electric and electrochemical properties [4]. According to Zhou et al., graphene modified electrodes exhibit similar potential window as graphite material (2.5 V in 0.1 M phosphate buffer, pH = 7.0) [11].

Amongst all derivatives of graphene, graphene oxide has attracted the biggest attention. It is relatively easy to prepare in large quantity via many oxidative approaches as reported by Hummers and Offeman [12], Stankovich [13] or lately Marcano et al. [14]. Thus prepared graphene oxide contains various functional groups such as hydroxyl, epoxide or carboxyl residues [9,15] that make it suitable for modifications of surfaces without any demanding additional processes [5]. On the other hand, for electrochemical purposes, further chemical or electrochemical reduction is usually needed. Thus prepared graphene or more precisely, reduced graphene oxide, contains structural defects and functional groups which can lead to partial loss/drop of the electron mobility [10,13,16]. In spite of the structural defects, appreciable advantage of graphene oxide is that this material contains less or even no metal impurities compared to its nearest structural competitor carbon nanotubes [17,18].

The majority of authors agrees that the electrochemical behaviour of graphene stems from the large surface, which is obtained due to the defect sites [11,19–23]. This principle is in accord with the Banks and Compton's work, who suggested, that catalytic activity of graphite based electrodes mostly comes from

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the edges and the defects in their structure [24]. Other authors study and describe catalytic activity caused by heterogeneous atoms due to modification approaches [7,25,26] and doping [27] or pH dependent catalysis [28].

Hydrogen peroxide is a typical product of oxidase based enzymatic reactions and a substrate for peroxidases, therefore its determination is still in the main focus. In comparison with other methods, electrochemistry offers fast, simple, sensitive and non-expensive way of its detection [6,11,29]. However, the effects of electrochemical interferences upon detection of H_2O_2 were still not reasonably solved. Ascorbic acid (AA) is a typical antioxidant which naturally occurs in fruits and vegetables. It is also very important for human health and is being widely used for the treatment of common cold, hepatic diseases and even cancer [30,31]. Dopamine (DA) is one of the major catecholamine neurotransmitters in mammalian central nervous, renal, cardiovascular and hormonal systems. Furthermore, it regulates cognition and emotions [6,22,28,32,33]. Since AA and DA are electroactive and widely present in body fluids, both represent major electrochemical interferences upon hydrogen peroxide detection and hinder electrochemical analysis of blood samples in general.

Majority of the graphene-related work describes its superior properties for electrochemical biosensing using graphene-modified electrodes for the rejection of effects of interfering compounds. During the study of the literature, we encountered strange inconsistencies in the reports on electrochemical behaviour of graphene. Lately several publications reported that graphene based electrodes have shown electrocatalytic activity towards diverse compounds such as H_2O_2, O_2, NADH, uric acid and much more [6,27,29,33–39]. In this report, we want to critically assess and clarify the seeming "electrocatalytic" effect (and specificity) of graphene towards hydrogen peroxide for further more effective utilisation in the electrochemical analysis.

2. Experimental

2.1. Chemicals

Graphite powder, 100 mesh (~150 μm) was obtained from Alfa Aesar (www.alfa.com). Hydrochloric acid, phosphoric acid, potassium permanganate, ethanol, diethyl ether, hydrogen peroxide and potassium chloride were purchased from Penta (www.penta-chemicals.eu) and sulfuric acid from Lach:ner (www.lach-ner.com). Ascorbic acid, dopamine hydrochloride, cysteamine hydrochloride and (3-aminopropyl)triethoxysilane (APTES) were obtained from Sigma-Aldrich (www.sigmaaldrich.com) and mica grade V-1 muscovite was purchased from SPI Supplies (www.2spi.com). Milli Q water was used throughout.

2.2. Preparation of graphene oxide

The samples of graphene oxide were synthesised according to the Improved Hummers method (IHM) by Marcano et al. [14] with some adjustments in acidity of the mixture, temperature and timing. Briefly, the mixture of 96% sulfuric acid and 85% phosphoric acid in 7:3 ratio (315/135 ml) was poured over the mixture of graphite and potassium permanganate in 1:5 ratio (3/15 g). The whole mixture was then stirred at 60 °C for 5 hours after which another 6 g of potassium permanganate were added to reach the final ratio of graphite/potassium permanganate 1:7 and the mixture was left under stirring for another 10 hours at 50 °C. The mixture was then cooled down to room temperature, poured over a mixture of ice and hydrogen peroxide (500 g ice and 10 ml of 30% hydrogen peroxide) and stirred. The colour of the mixture changed into bright orange. Filtration over 300 μm sieve followed in order to remove bigger particles (similarly to IHM); after centrifugation at 8 000 rpm for 15 min, the remaining solid was washed with water (2x), 30% hydrochloric acid (2x), ethanol (2x) and finally transferred into beaker, diluted in 300 ml of diethyl ether and filtered through 0.2 μm Teflon© filter under vacuum and let dry overnight under vacuum.

Thus prepared graphene oxide was then diluted in acidified water pH 3.0, sonicated for 2 hours and centrifuged for 20 minutes at 6 000 rpm in order to separate any multi-layered particles from the solution. The remaining gold-brownish solution was then used for experiments and stored at 4 °C.

2.3. Characterisation of graphene oxide

2.3.1. Inductively coupled plasma – mass spectrometry

Samples were analysed by inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS, Agilent Technologies) after sample decomposition in microwave digestion system (MWS3+, Berghof) with concentrated HNO_3, H_2O_2 and HCl (Merck, analytical grade chemicals). Content of Mn and Fe was quantified by external calibration with correction on 72Ge (internal standard). Semi-quantitative analysis was also performed on samples and content of other metals (more than 50 metals were measured).

2.3.2. Fourier-transformed infrared spectroscopy (FTIR)

FTIR spectra were measured on Bruker Tensor 27 (spectral range 4000–400 cm^{-1}, resolution 4 cm^{-1}) equipped by Diamond-ATR accessory device. IR spectra were evaluated with the OPUS 7.2 software.

2.3.3. Raman spectroscopy

Raman spectra were recorded using micro-Raman spectrometer Horiba Labram HR Evolution with a 532-nm laser as an excitation source. Samples were analysed in dry state on a microscope slide.

2.3.4. Atomic force microscopy (AFM)

Atomic force microscope Dimension FastScan (Bruker, Santa Barbara, CA USA) was used to measure the topography of the prepared graphene oxide. The FastScan-A probe (Bruker) with spring constant 18 N/m and resonance frequency of cantilever 1400 kHz was used for the imaging.

Samples were prepared as follows. The mica squares (1.5 cm × 1.5 cm) were cleaned with a two-sided adhesive tape and any remaining microelements were removed by compressed air. A drop (5 μl) of graphene oxide solution was deposited on the freshly cleaned mica surface at room temperature. Scanning of the sample was initiated after drying of the droplet on the mica surface in air.

2.3.5. Characterisation of the gold electrodes

Gold electrodes were characterised by laser ablation. Analyses were conducted using a double focusing ICP-MS Element 2 (Thermo Scientific, Bremen, Germany) with an attached Analyte G2 laser ablation system (Photo Machines Inc., Redmond, WA, USA). No significant impurities were found.

2.4. Preparation of the electrodes

Gold electrodes (Au disk 0.5 mm in diameter, embedded in glass) were cleaned prior to their modification. The electrodes were immersed into a freshly prepared piranha solution (3:1) for one hour, then in chromosulfuric acid for 30 min, washed with water and polished to mirror-like finish using a sequence of 0.3 and 0.05 μm alumina slurries and finally sonicated for 10 min in ethanol in order to remove any remaining particles from polishing, and dried in a nitrogen stream.