A new route for the integration of a graphene/diazonium/PEDOT electrode towards antioxidant biomarker detection

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ABSTRACT

A new route for the integration of a graphene-based electrode on a silicon substrate was studied for the simultaneous detection and assay of ascorbic (AA) and uric (UA) acids. Graphene was synthesized by catalytic chemical vapour deposition (CVD) from methane as carbon feedstock onto both platinum foils and supported thin platinum films on silicon wafers which were used as catalyst. Graphene was characterized by Raman spectroscopy and electron microscopies. The resulting electrodes were further functionalized by successive electrodeposition of a stabilizing diazonium layer and a poly(3,4-ethylenedioxythiophene) (PEDOT) conductive film. AA and UA determination on such modified electrode was performed either by cyclic voltammetry or by differential pulse voltammetry. In the latter case, the selectivity was about 380 mV and the sensitivity was 2.40 and 2.75 $\mu A\ cm^{-2}\ \mu M^{-1}$ for AA and UA, respectively. The present study shows that monolayer graphene allows a greater sensitivity of AA and UA detection than that observed with modified glassy carbon electrode.

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1. Introduction

Oxidative stress results from an imbalance between the production of highly oxidizing species and the antioxidant defense system of human body [1]. Particular attention has been paid to this biochemical process over the last two decades [2,3], mainly because of its presumed implication in early stages of pathologies such as cataract, cancer, cardiovascular and neurodegenerative diseases [4–10]. The antioxidant defense system is composed of a set of enzymes and low molecular weight compounds. Among the latter, ascorbic (AA) and uric (UA) acids are of particular interest. Indeed AA is one of the most powerful hydrophilic antioxidant whereas UA concentration in serum is presumably a major marker of specific diseases that may induce heart or kidney problems [11,12]. Moreover, both AA and UA are present in numerous biological fluids (plasma, serum, urine, tears) and are therefore reliable biochemical markers for the detection of the above-mentioned pathologies. Numerous methods exist to quantify AA and UA in body fluids, in particular enzymatic methods [13], spectrofluorometry [14], high-performance liquid chromatography (HPLC) analysis [15], or capillary electrophoresis [16], but they are expensive and time-consuming. Electrochemical sensors [17] could represent a suitable alternative due to their advantages such as low cost, fast analysis and reliability [18–20]. Sensors' performances, namely sensitivity, selectivity, limit of detection and lifetime, can be drastically improved by the functionalization of the electrode surface. In this way, the potentialities of electrografted diazonium salts have been studied in recent works since they combine a strong covalent bonding with the electrode surface and a good versatility for further functionalization [21–24]. In particular, the additional electropolymerization of 3,4-ethylenedioxythiophene (EDOT) has proved to be an efficient way to enhance the selectivity of the electrodes [21,25,26]. Several electrode materials have been considered in previous works such as glassy carbon [21] or gold [27]. Carbon-based electrodes are particularly well-suited in terms of versatility and cost-effectiveness: in particular, glassy carbon has shown an exceptionally good behavior for such applications [20,22,26,27]. However, this material is hardly compatible with the silicon technologies required for a cost-effective mass production in series of integrated microelectrodes and the associated electrochemical microcells [28,29]. On the other hand, graphene is a two-dimensional carbon structure which is used in a growing number of applications because of its high electrical conductivity, large specific surface area and excellent electrochemical

stability [30]. It can be produced at reduced costs with a good quality by the catalytic chemical vapour deposition (CVD) process, thus being compatible with silicon technologies [30]. Moreover, graphene has shown high performances in electroanalysis and electrochemical detection of small molecular targets such as hydrogen peroxide or glucose, and nucleic acids [31,32]. Platinum is a good catalyst for graphene synthesis because of the low carbon solubility in Pt (0.005 wt%) close to the deposition temperature (1000 °C) [34], leading to mono-layer graphene. In particular, crystalline graphene mono-layers can be synthesized by catalytic CVD on platinum foils [33–36]. Moreover, the high melting point, low thermal expansion coefficient and high inertness of Pt provide a smooth graphene morphology. Lastly, after annealing Pt over 600 °C, the (111) orientation dominates, thus inducing very weak graphene-substrate interactions. Consequently platinum has a quite limited effect on the physical properties of graphene. It has even been shown that the electronic structure of the graphene grown on Pt is nearly the same as that of the free-standing graphene [33]. On another hand, Pt is a well-known conductive material and has been thoroughly used for the fabrication of microelectrodes [37,38]. Furthermore it is fully compatible with silicon microtechnologies.

The present study constitutes the first step for the conception of a mass production of microsensors for AA and UA assay. It aims at developing the proof-of-concept of a mixed 4-thiophenylbenzene diazonium (TBD)/poly(3,4-ethylenedioxythiophene) (PEDOT) functionalized graphene electrode integrated on silicon substrate for the simultaneous detection and assay of UA and AA. The protocol of CVD graphene synthesis on (i) Pt foils and (ii) Pt thin films, were first presented, together with the electrochemical functionalization by both TBD salt grafting and EDOT electropolymerization. The Raman spectroscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses of the resulting modified electrodes were detailed. Both selectivity and sensitivity for AA and UA oxidations were finally evaluated by electrochemical methods.

2. Experimental

2.1. Chemicals

All products were used as received. Reactive gases for the CVD processes (Ar alpha 1, H_2 alpha 1 and CH_4 N45) were supplied either by Air Liquide or Linde. 4-thio(phen-2-yl)aniline (TPA) ($C_{10}H_9NS$), 3,4-ethylenedioxythiophene (EDOT) ($C_6H_6O_2S$), sodium nitrite ($NaNO_2$), tetrabutylammonium hexafluorophosphate (Bu_4NPF_6), ascorbic acid (AA) ($C_6H_8O_6$, 99% powder) and uric acid (UA) ($C_5H_4N_4O_3$, 99% powder) were purchased from Sigma-Aldrich. Potassium dihydrogenophosphate (KH_2PO_4), di-potassium hydrogenophosphate (K_2PHO_4) and acetonitrile (ACN) (extra dry) were supplied by Acros Organics. Phosphate buffer saline (PBS) solution (0.1 mol L^{-1}, pH 7) was prepared by mixing dipotassium phosphate and monopotassium phosphate in Milli-Q water (18 MΩ cm). Acid solutions were prepared by dilution of 37% hydrochloric acid (HCl) from VWR using Milli-Q water.

2.2. Catalytic substrates

The graphene synthesis process was first developed using 100-μm thick Pt foils (from Mateck, 99% in purity). In order to demonstrate the feasibility of microelectrodes production in series, Si(100)/SiO$_2$/Ta/Pt stacks were fabricated from oxidized silicon wafers with a SiO$_2$ thickness of 600 nm. Thin metallic layers (10 nm Ta, 100 nm Pt) were deposited by sputtering in a conventional UNIVEX 450D physical vapor deposition (PVD) reactor. Tantalum was used as both an adhesion and a barrier layer for Pt on SiO$_2$ considering the high temperature required by the subsequent graphene deposition process. All experiments were conducted with samples of 2×2 cm^2.

2.3. Chemical vapour deposition

The catalytic CVD of graphene was achieved in a home-made CVD reactor consisting in a horizontal cylindrical quartz tube of 1000 mm length and 90 mm inner diameter, cooled at each extremity by water circulation. A gas injection/pumping system enabled the circulation of reactive gases and the extraction of unreacted species and by-products. A 760-mm long furnace composed by three heating zones surrounded the tube. Quartz sample-holders were used to maintain the samples in the reactor. The catalytic substrates were placed in the central isothermal part of the heating zone. The full description of the home-made reactor is provided as Supplementary materials Fig. S1 (see e.g. [39]). Graphene was obtained through three successive steps under low pressure Ar/H_2 reductive atmosphere (850 and 50 standard cubic centimeters per minute (sccm) respectively). The first step was a thermal pretreatment of the catalytic surface: the substrate was heated from 25 to 1045 °C under low pressure (2.1 Torr) and maintained at 1045 °C for 15 min. The second step consisted in increasing the pressure up to 700 Torr and in introducing 5 sccm of methane (carbon precursor). CH_4 flow and heating were turned off after either 12 or 24 min. Finally the substrate was cooled down from 1045 to 25 °C at about 10 °C min^{-1}.

2.4. Graphene characterization

Raman spectroscopy was performed using a HORIBA Jobin Yvon's LabRAM 800HR spectrometer with a 532-nm wavelength laser source.

Scanning electron microscopy analyses were performed using a JEOL 7600 microscope equipped with an Oxford Link ISIS 300 EDX analyzer.

Transmission electron microscopy and diffraction were carried out using a CM30 Philips TEM equipped with a LaB$_6$-gun and operated at 150 kV while taking care of limiting electron damaging. The TEM specimens were prepared as follows: the graphene layer was protected by a spin coated 500-nm PMMA film. Then H_2 was bubbled between the graphene layer and the Pt host substrate by applying a 2 mA current in a 1 M NaOH solution, according to a previously reported procedure [33,40]. The sample was subsequently transferred to a TEM copper grid (200 mesh) coated with amorphous lacey carbon film allowing for its observation. Note that regarding the graphene synthesized on Pt thin films, the insulating SiO$_2$ interlayer did not allow to take a good electrical backside contact for applying the current, rendering the transfer of graphene delicate. Therefore, only graphene synthesized on Pt foils was characterized by TEM.

2.5. Electrochemical instrumentation

All the electrochemical experiments were performed at room temperature using a Metrohm μ−Autolab II potentiostat interfaced to a personal computer and controlled with GPES 4.9 software package. A conventional three-electrode cell was used with a Pt wire and an Ag/AgCl/KCl (3 M) ($E° = +0.21$ V vs. NHE) electrode connected to the cell by a capillary as counter and reference electrodes, respectively. All the potentials are expressed with respect to this reference. The working electrode was either the unmodified or modified graphene electrode. The working electrodes were either a 5.5 mm-diameter disk ($A = 23.8$ mm^2) or a 1×1 cm^2 square ($A = 1.0$ cm^2) for Pt foil and Pt thin film electrodes, respectively.

2.6. Electrochemical functionalization of the graphene electrode

Unless otherwise stated, the solutions were deaerated prior to be used by nitrogen during 10 min. Prior to each modification step, the graphene electrode was cautiously washed with Milli-Q water and ethanol. 4-thiophenylbenzene diazonium (TBD) was prepared according to the following procedure. A cold solution of $NaNO_2$ (0.94 mg, 13.9 mmol) in Milli-Q water (1 mL) was slowly added to a 4 mL ice-cold solution of 0.1 M HCl containing TPA (2.19 mg, 12.4 mmol). The mixture was left to