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A New Route for the Integration of a Graphene/Diazonium/PEDOT Electrode towards Antioxidant Biomarkers 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 differential pulse voltammetry. In the latter case, the selectivity was about 380 mV and the sensitivity was 2.40 and 2.75 $\mu\text{A cm}^{-2} \mu\text{M}^{-1}$ 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.

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 [5–10]. 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 CVD synthesis results of graphene on (i) Pt foils and (ii) on 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, H₂ alpha 1 and CH₄ N45) were supplied either by Air Liquide or Linde. 4-thio(phen-2-yl)aniline (TPA) (C₁₀H₉NS), 3,4-ethylenedioxythiophene (EDOT) (C₆H₆O₂S), sodium nitrite (NaNO₂), tetrabutylammonium hexafluorophosphate (Bu₄NPF₆), ascorbic acid (AA) (C₆H₈O₆, 99 % powder) and uric acid (UA) (C₅H₄N₄O₃, 99 % powder) were purchased from Sigma-Aldrich. Potassium dihydrogenophosphate (KH₂PO₄), di-potassium hydrogenophosphate (K₂PHO₄) 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)/SiO₂/Ta/Pt stacks were fabricated from oxidized silicon wafers with a SiO₂ 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 SiO₂ considering the high temperature required by the subsequent graphene deposition process. All experiments were conducted with samples of 2x2 cm².

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 **Figure S1** (see e.g. [39]). Graphene was obtained through three successive steps under low pressure Ar/H₂ 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). CH₄ flow and heating were turned off after either 12 or 24 minutes. 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 LaB₆-gun and operated at 150 kV while taking care of limiting electron damaging. The TEM specimens were prepared as follows: the graphene layered was protected by a spin coated 500-nm PMMA film. Then H₂ was bubbled between the graphene layered 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 SiO₂ 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) ($E^\circ = +0.21$ 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 ($A=23.8$ mm²) or a 1x1 cm² square ($A=1.0$ cm²) 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 NaNO₂ (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 react at 4 °C for 10 min and then transferred into the electrochemical cell. Diazonium grafting onto graphene was achieved using cyclic voltammetry (CV) by recording 10 scans between 0.6 V and -0.4 V at 100 mV s⁻¹.

Poly(3,4-ethylenedioxythiophene) (PEDOT) was generated by recording 10 cyclic voltammograms (CVs) between 0.8 V and 1.5 V at 50 mV s⁻¹ in a 0.1 M Bu₄NPF₆ acetonitrile solution containing 2.5 mM EDOT. The parameters used were those defined and optimized in a previously reported work from our group [21]. After each modification step, the electrode was carefully rinsed with Milli-Q water and ethanol in order to remove weakly adsorbed moieties.

2.7. Electrochemical detection of ascorbic and uric acids

The electrochemical detection of AA and UA on the functionalized graphene electrode was investigated in 0.1 M PBS solution (pH 7) containing an equimolar mixed solution of 200 μM AA and UA by cycling voltammetry and differential pulsed voltammetry at a scan rate of 50 mV s⁻¹. In the former case the potential range used was between -0.2 V and 0.6 V. In the latter case the potential range used was between -0.2 V and 0.5 V and the following optimized parameters were used for the waveform: modulation time: 0.01 s; interval time: 0.1 s; modulation amplitude: 0.05 V; step potential: 0.005 V.

3. Results and discussion

The steps used to fabricate the graphene-based electrode are summarized in **Figure 1**.

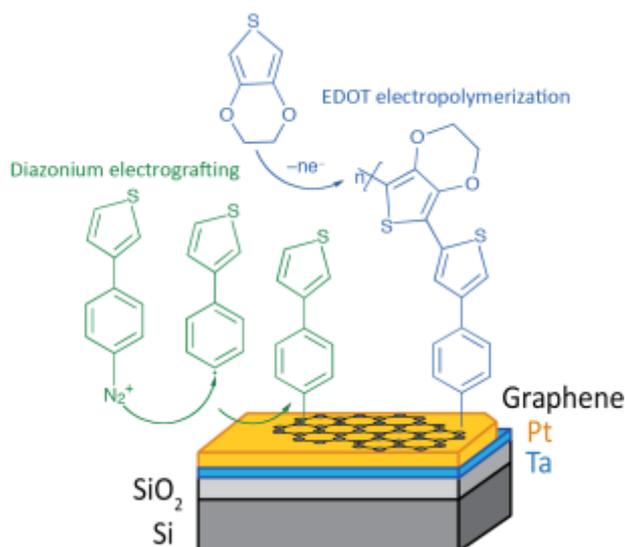


Figure 1. Schematic of the graphene-based electrode elaboration and functionalization consisting in four successive steps: i) deposition of a thin Pt film onto a Si(100)/SiO₂/Ta wafer, ii) synthesis of graphene by catalytic chemical vapour deposition, iii) diazonium electrografting and iv) EDOT electropolymerization.

3.1. Synthesis of graphene by chemical vapour deposition

Preliminary CVD experiments were conducted on 100- μm thick Pt-foils in order to obtain high quality graphene. The optimized synthesis parameters were temperature, methane concentration, pressure and deposition duration. Once the best synthesis conditions were determined on Pt foils (see Experimental Section for detail), these parameters were applied for graphene synthesis on Ta/Pt thin films. The resulting graphene was characterized again in order to ascertain the good quality of the layer for these experimental conditions.

Figure 2 shows SEM micrographs of graphene flakes obtained on both Pt foils (**Figures 2a,b**) and Pt thin films (**Figures 2c,d**) using the same synthesis parameters (12 min of deposition, 5 sccm of methane). Note that it was observed that for a CH₄ flow rate higher than 5 sccm, graphite agglomerates covered the sample surface. On **Figures 2a,b** large flakes of 0.03 mm² and smaller ones of about 25 μm^2 were observed. A hexagonal shape, characteristic of monocrystalline graphene, was clearly visible for the smaller ones. On

Figures 2c,d graphene flakes of different sizes close to each other were observed over the entire surface of the thin platinum film. They form an almost continuous graphene-like layer of several cm^2 over the entire surface of the thin platinum films. The contrast variation between the flakes was due to the difference of graphene number of layers. Moreover bright and drop-shaped areas underneath the graphene layered were also noticed, as a consequence of a slight dewetting of the Pt layer due to the high CVD process temperature. Preliminary experiments carried out using Ti as an adhesion layer instead of Ta led to a total dewetting of the Pt layer (see supplementary materials **Figure S2**), probably because of the high temperature treatment combined with the reductive H_2 atmosphere in the reactor. Thus, the Ta layer was effective to stabilize the Pt layer.

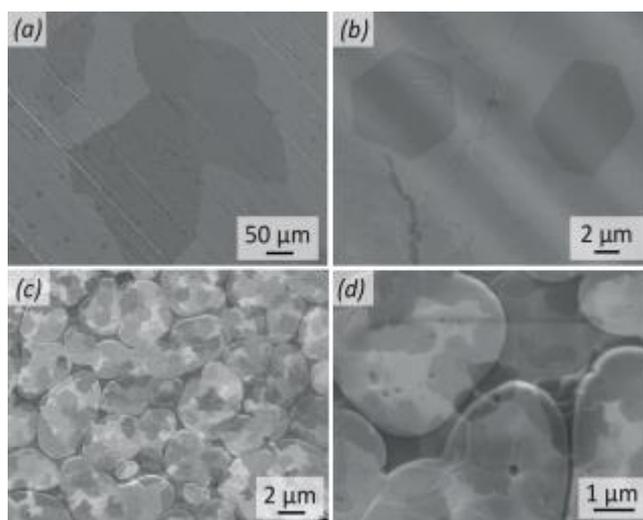


Figure 2. SEM micrographs of graphene flakes synthesized by catalytic chemical vapor deposition on (a,b) a 100- μm thick Pt foil and on (c,d) a 100-nm Ta/Pt thin film deposited on a Si/SiO₂ wafer.

Figure 3 displays the Raman spectra of graphene deposited during 12 (**Figure 3a**) and 24 min (**Figure 3b**) on Pt foils and during 12 min on Pt thin films (**Figure 3c**). For each surface, analyses were performed on at least six different areas in order to allow a statistical evaluation of the graphene quality. These analyses confirmed that graphene was continuous on all the samples.

Raman spectroscopy is commonly used to validate the presence of graphene, as well as to characterize both its structural quality and the number of graphene monolayers. Several features of the resulting spectra are well-known to be considered for this purpose (e.g. see supplementary materials **S3**), in particular considering the different band intensities noted I_D , I_G , and I_{2D} respectively [41,42].

Figure 3 shows that each sample presented the characteristic D and G bands of graphenic carbons, and an intense band in the frequency range of the characteristic $2D$ band. It has to be noted that for the spectra of **Figure 3a** and **3b**, the peak located in the $2D$ band region ($\sim 2700\text{ cm}^{-1}$) presents one or two shoulders and exhibits shifts (of 100 cm^{-1} and 50 cm^{-1} on **Figure 3a** and **Figure 3b**, respectively) towards higher wave numbers for graphene synthesized on Pt foils. This phenomenon is not totally elucidated but it could be assigned to C–H stretching modes or to constraints present in the graphene layer [43]. The 12-minute synthesis on Pt thick foil (**Figure 3a**) led to a multi-layer graphene with defects ($\frac{I_D}{I_G} = 0.57$), whereas increasing the deposition duration up to 24 min on the same substrate (**Figure 3b**) and in the same temperature and methane partial pressure conditions led to graphene multi-layer with lower defect concentration ($\frac{I_D}{I_G} = 0.21$). Thus, increasing the graphene CVD duration induced a higher number of graphene layers according to a lower I_{2D}/I_G ratio, but with lower defect concentration. This result is in good agreement with those of Lee and Jeong [36]. Our aim in increasing the deposition duration was to reduce the defect concentration by annealing effect and by substrate surface modification, which seems to be verified. The most often assumed mechanism of graphene formation involves a dissolution of carbon into the metal and a segregation of carbon on the metal surface during the cooling step [33]. According to the work by Lee and Jeong [36], a mechanism of graphene formation by surface adsorption could also co-exist, thus explaining that the number of graphene layers increased with the deposition duration.

Since Pt thin films are smoother than Pt foils, the 12-minute long CVD process was chosen for the synthesis of graphene on thin Pt films. The graphene surface obtained (**Figure 3c**) presented a very low *D* band intensity. The corresponding ratio of band intensities $\frac{I_D}{I_G}$ was equal to 0.1 which corresponded to a low defect concentration, whereas the $\frac{I_{2D}}{I_G}$ is equal to 2.45 with a sharp and symmetric *2D* band, which corresponds to monolayer graphene. Furthermore, the full width at half maximum (FWHM) of the *G* band was particularly low, confirming the high quality of graphene. Additionally, the FWHM of the *2D* band was evaluated for several measurements performed on different points over the substrate surface (insert in **Figure 3c**). It was close to 31 cm^{-1} which could correspond to monolayer graphene in turbostratic packing stacking. The low deviation (< 10%) of the FWHM values for the several points assessed the uniformity of the graphene layer over the substrate in term of number of layers [44]. The graphene quality in terms of layer number and of defects was thus better on Pt thin films than on Pt foils. This can be explained by the fact that the Pt foils present a non-negligible intrinsic surface roughness whereas the Pt films formed by PVD are very smooth.

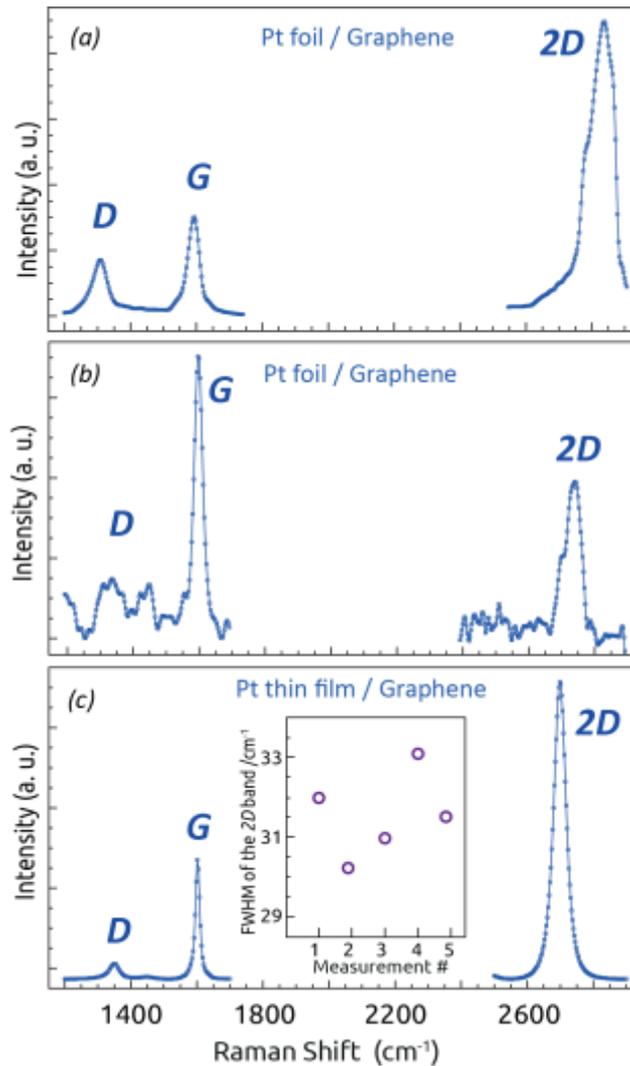


Figure 3. Raman spectra of graphene synthesized by catalytic chemical vapour deposition (a,b) on a 100- μm thick Pt foil and (c) on 100-nm Pt thin film deposited on a Si(100)/SiO₂/Ta wafer. The CVD duration was 12 min (a,c) and 24 min (b). Insert of (c): FWHM of the 2D band at different locations over the substrate for the graphene layer synthesized on Pt thin film.

To further characterize the graphene quality, the samples were analyzed by transmission electron microscopy (TEM). **Figure 4** displays the resulting micrographs and diffraction patterns. Low magnification images (**Figure 4a**) confirmed that graphene was continuous over large area domains. The domains exhibited wrinkles that probably originated from the deposition process on the TEM grid. Thanks to the wrinkles, high-resolution images (**Figure 4b**) showed that the domains were actually not mono-layered graphene but multi-layered graphene, since they were made of less than 10 stacked graphene (a maximum of 5 in

the figure) with good nanotexture (i.e., distortions are little). Finally, the diffraction patterns (**Figure 4c**) exhibited two sets of six sharp spots concentrically dispatched according to a hexagonal symmetry, which is the mark of either single-layer graphene or single graphite crystal, when lying flat. As the graphene here were multi-layered (**Figure 4b**), the single graphite nature was ascertained (i.e., all the graphene in the stack were coherently piled up according to the ABAB Bernal sequence), at least in the dimension range ($\sim 1\mu\text{m}$) of the opening of the selection aperture used for obtaining the diffraction patterns [42]. In addition, the spot sharpness was consistent with large, micron-size mono-domains. Note that the transferring step of graphene to the TEM grid could imply a relaxation of constraints. To summarize, the TEM investigation showed that the graphene domains grown on Pt foil for 12 minutes were high quality graphene stacked by less than ten layers as micron-sized single graphite crystals.

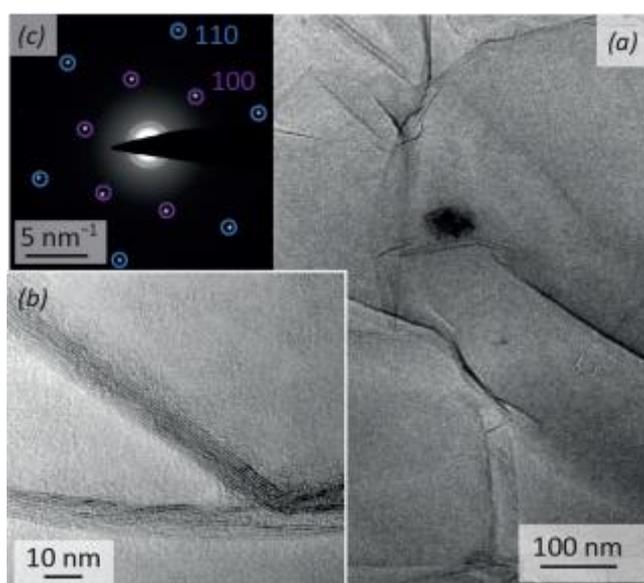


Figure 4. (a) TEM micrograph (plane view) of a free-standing graphene sheet synthesized by catalytic chemical vapour deposition on a Pt foil for a 12-min deposition process, (b) HRTEM cross-section view (wrinkle) of a multi-layered graphene sheet, (c) electron diffraction pattern obtained from a single, multi-layered graphene flake in an area free of wrinkles, hence showing the graphite structure (see text).

3.2. Graphene electrode functionalization

The functionalization of the graphene-based electrode was performed by diazonium electrografting followed by EDOT electropolymerization in a coupled approach (**Figure 1**). Graphene electrodes elaborated either on Pt foils or on Pt thin films supported on oxidized Si wafers were investigated.

In order to evaluate the graphene stability under the electrochemical conditions, 50 consecutive CVs were first recorded in a 0.1 M PBS (pH 7) solution. **Figure 5a** displays the 1st, 25th and last of 50 consecutive scans monitored on a graphene electrode supported on a Pt thin film on oxidized wafer. The resulting CVs showed a classical shape with a reduction peak at 0.05 V corresponding to O₂ reduction and the typical H₂ evolution around -0.6 V. The system exhibited a stable behavior as a function of time since no evolution of the CVs was noticed between the 1st and the 50th scan. Note that the same experiment was conducted on graphene electrode supported on Cu substrates (not shown). In this case, an ohmic contribution increasing with the number of scans was observed. This was attributed to the oxidation and poisoning of the Cu electrode surface by adsorption of reaction by-products, leading to a lower conductivity. **Figures 5b,c** show CVs recorded in a 0.1 M HCl solution containing 2.5 mM TBD on Pt foil/graphene and Pt thin film/graphene electrodes, respectively. On the former electrode (**Figure 5b**), a cathodic peak located at -0.15 V was observed, which corresponded to the diazonium reduction and subsequent grafting onto the graphene surface. It has to be noticed that the typical reduction peak decay and cathodic shift were very progressive all along the 10 consecutive scans, indicating a slow grafting kinetics probably due to the graphene flatness. Conversely, on the Pt thin film/graphene electrode (**Figure 5c**), the self-inhibiting behavior of the grafted organic layer was observed from the second scan, suggesting a faster grafting kinetics in this latter case. Moreover, a reduction

pre-peak was noticed around 0.06 V together with the reduction peak at -0.15 V, in accordance with a diazonium reduction process easier on the Pt thin film/graphene than on the Pt foil/graphene. However and except in the case of 4-nitrobenzene diazonium [22], the exact mechanism involved in the appearance of this pre-peak remains unclear. The graphene electrodes modified by diazonium grafting were further functionalized by 3,4-ethylenedioxythiophene (EDOT) electropolymerization *via* cyclic voltammetry. **Figure 5d** shows the 1st, 5th and 10th corresponding scans. In accordance with a previously reported work performed on GC electrodes, the monomer oxidation started around 1.3 V and the corresponding current increased all along the electropolymerization process as the result of the polymer formation and subsequent increase of the active surface area [21].

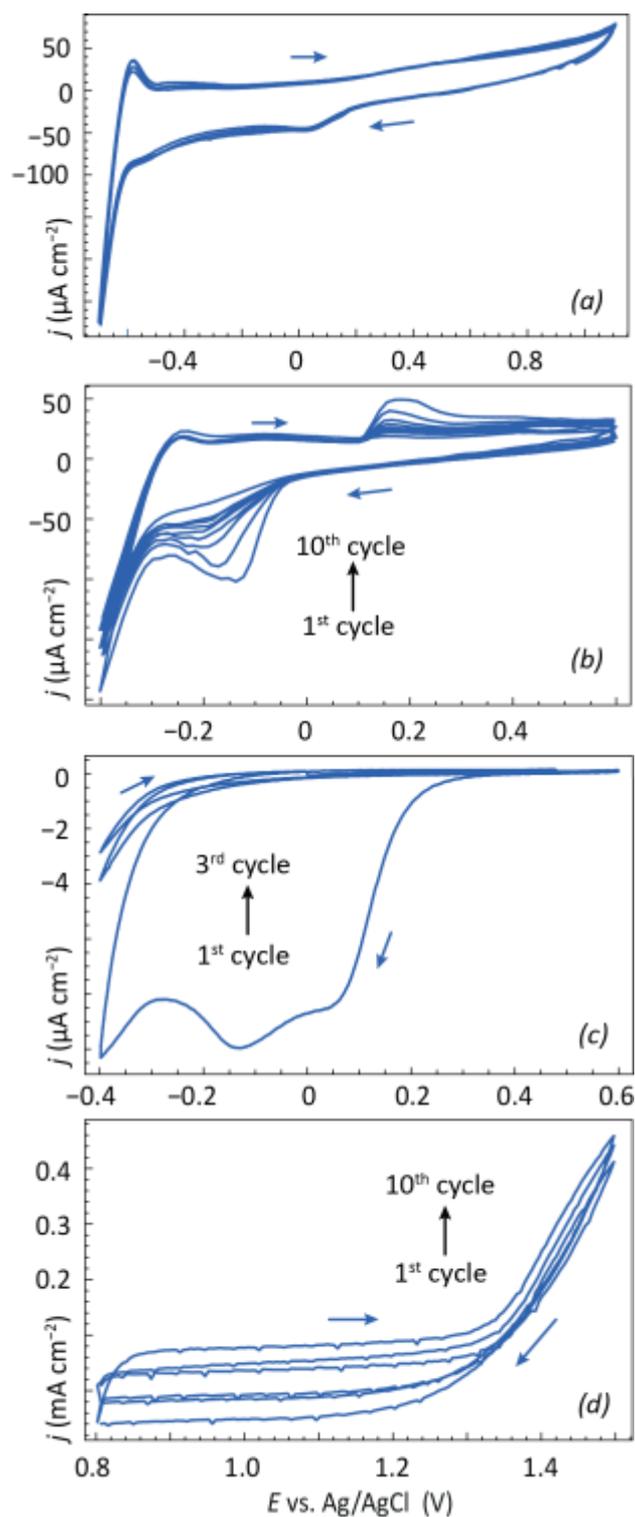


Figure 5. (a) CVs recorded on a Pt thin film/graphene electrode in a 0.1 M PBS (pH 7) solution. The 1st, 25th and 50th cycles are shown. Scan rate: 50 mV s⁻¹. CVs recorded in a 0.1 M HCl solution containing 2.5 mM TBD on Pt foil/graphene (b) and on Pt thin film/graphene electrodes (c). Scan rate: 100 mV s⁻¹. (d) CVs recorded in a 0.1 M Bu₄NPF₆ ACN solution containing 2.5 mM EDOT on a Pt thin film/graphene electrode previously functionalized by diazonium grafting. The 1st, 5th and 10th cycles are shown. Scan rate: 50 mV s⁻¹.

3.3. Biomarkers detection

The diazonium/PEDOT functionalized graphene-based electrode was used for the simultaneous detection of uric and ascorbic acids. **Figure 6** presents CVs recorded in a 0.1 M PBS solution (pH 7) containing a 200- μ M mixed AA and UA solution. **Figure 6a** displays the three successive scans performed on a Pt foil/graphene/diazonium/PEDOT electrode. The two anodic peaks observed on the forward scan and located at 0.05 and 0.4 V were assigned to AA and UA oxidation, respectively. The shapes of these peaks were consistent with a diffusion-controlled process for AA and an adsorption-limited one for UA, in accordance with previous studies [21,45]. However, it has to be noticed that both peaks exhibited a slightly resistive behavior which was not observed on glassy carbon (GC) material [21]. The peak separation was about 350 mV, demonstrating the high selectivity of the electrode towards the two analytical targets. As a comparison, GC modified electrode obtained using the same mixed surface functionalization methods (i.e. TBD and EDOT deposited by cyclic voltammetry, see section 2.6) exhibited an analytical response very similar to that of bare GC with an oxidation peak at 0.6 V assigned to UA coupled with a shoulder around 0.25 V assigned to AA [21-fig. 2]. No real peak separation between UA and AA was observed in these two former cases. This observation comforts graphene as a good alternative to GC (modified or not) in terms of selectivity and sensitivity towards AA and UA. Additionally, a recent review [46] has reported that modified glassy carbon electrodes usually exhibit a AA–UA peak separation in the range 0.14–0.21 V, whereas using graphene as interlayer systematically leads to a peak separation higher than 0.2 V. In the present study, the peak separation was in the range of 0.35 V. This evidences the excellent ability of our electrode to improve the selective detection of AA and UA, and further support the enhancement of the electroanalytical response due the graphene interlayer. Furthermore, it has to be noticed that the mixed diazonium/PEDOT interface has proved to be much more stable than PEDOT

without diazonium [21]. However, a rapid decrease in the amperometric responses was observed here after three successive scans, since AA oxidation peak current decreased from 150 to 40 $\mu\text{A cm}^{-2}$ (73 % loss) and UA one decreased from 250 to 90 $\mu\text{A cm}^{-2}$ (64 % loss). Although by-products coming from the AA and UA oxidation could inhibit the detection sites of the electrode, the reason for this phenomenon is not clearly understood yet. However it was not observed on GC electrode. One may then assume that the Pt foil/graphene interface induced a different structuration of the organic layer, altering the conduction properties of the PEDOT film upon cycling that led to such a peculiar behavior. Note that the selectivity of the electrode for the simultaneous detection of UA and AA remained unchanged over the scans while peak currents decreased. Similar experiments were carried out on the Si/Pt thin film/graphene/diazonium/PEDOT electrode supported on oxidized Si wafer (**Figure 6b**). In this latter case, AA and UA peaks were slightly better separated (ca. 380 mV), and the resistive behavior noticed on the Pt foil/graphene/diazonium/PEDOT electrode was no longer observed. These observations supported the assumption of a beneficial structuration of the diazonium layer on the Si/Pt thin film/graphene leading to an enhanced conductivity and to a better sensitivity of the electrode.

Moreover the amperometric response was much more stable upon cycling on the Si/Pt thin film/graphene/diazonium/PEDOT electrode since the decrease in peak current after the 3 consecutive scans was only < 1 % and 6 % for AA and UA, respectively. Thus, the use of the Pt thin film as graphene substrate afforded a quite good structuration of the electrode functionalization, this latter being suitable for selective and repeatable AA and UA detection. This could play an important role in PEDOT nanostructuration leading to a signal enhancement as compared to GC modified electrode and a higher AA–UA peak separation. This results also in a favorable electron transfer kinetics and electrocatalytic activity toward

the oxidation of AA and UA of our graphene/diazonium/PEDOT modified electrode (**Figure 6**) again as compared to the GC electrode functionalized in the same conditions [21].

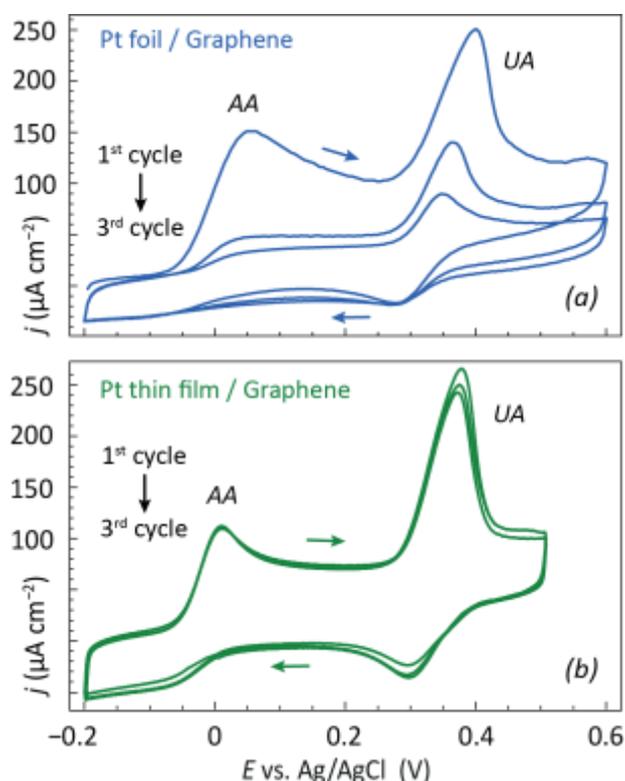


Figure 6. Successive cycles of a CV recorded on a functionalized (a) Pt foil/graphene/diazonium/PEDOT electrode and (b) Si/Pt thin film/graphene/diazonium/PEDOT electrode in a PBS 0.1 mol L^{-1} pH 7 solution containing $200 \mu\text{M}$ mixed AA and UA. Potential scan rate: 50 mV s^{-1} .

In order to further improve the sensitivity of the modified electrode, measurements were recorded in a AA and UA mixture solution by differential pulsed voltammetry (DPV) using the Si/Pt thin film/graphene/diazonium/PEDOT electrode. **Figure 7** shows the simultaneous detection of UA and AA. In this case, the sensitivity for AA detection was about $2.40 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ at a potential of -5 mV whereas it reached $2.75 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ for UA detection at a potential of 380 mV again enhanced with respect to GC/diazonium/PEDOT electrodes. In optimized conditions, this latter exhibited a sensitivity of 0.665 and $0.345 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$ for AA and UA, respectively [21]. This shows again the high sensitivity

and selectivity of the graphene-based electrode. It has to be noted that the sensitivity measurements reported in [21] were performed by linear regression for various AA/UA ratios (see e.g. [46] for a quite exhaustive review of the different types of electrodes and functionalization methods for AA/UA detection and their performances).

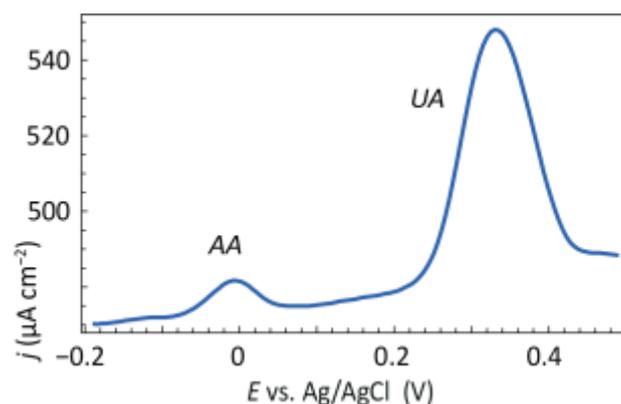


Figure 7. Differential pulsed voltammograms of simultaneous UA and AA detection on a functionalized Si/Pt thin film/graphene/diazonium/PEDOT electrode. A 200-μM mixed solution of UA and AA was used. Scanning rate: 50 mV s⁻¹.

4. Conclusions

In this work, we described the entire process for the synthesis of a graphene-based electrode on thin Pt films deposited on oxidized Si wafer in order to further develop a low cost integrated micro-device produced in series. The results showed that optimized graphene was obtained for a CVD process of 12 min at 700 Torr and 1045 °C. The graphene electrode was then functionalized by a stabilizing diazonium layer coupled to a conductive PEDOT layer that confers improved selectivity to the electrode. The resulting electrode was tested for the simultaneous detection of AA and UA. A high sensitivity of 2.40 and 2.75 μA cm⁻² μM⁻¹ was obtained for AA and UA respectively and the selectivity was about 380 mV. This measurement was performed for an equimolar mixed solution of 200 μM AA and UA. This value was close to that

obtained on classical GC electrode. We thus demonstrated the proof-of-concept for the feasibility of fabricating an integrated graphene-based electrode as a basis for future selective and sensitive sensor dedicated to biomarkers. Current works focus on the integration of this electrode into a final micro-device.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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