Electrochemical ascorbic acid sensor based on DMF-exfoliated graphene†

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This paper describes the electron transfer properties of graphene nano-sheets (GNSs) immobilised on pyrolysed photore sist film (PPF) electrodes. The former are produced by the dispersion and exfoliation of graphite in dimethylformamide, and they are characterised using transmission electron microscopy, scanning electron microscopy and Raman spectroscopy. Cyclic voltammetry and electrochemical impedance spectroscopy are used to quantify the effect of the GNSs on electrochemical surface area and on electron transfer kinetics. Compelling evidence is reported in relation to the importance of edge-plane sites and defects in the promotion of electron transfer at carbon nanostructures. A novel ascorbic acid (vitamin C) sensor is presented based on the PPF/GNS system, which is effective in the range 0.4 to 6.0 mM, with a 0.12 mM detection limit. The selectivity of the sensor is demonstrated using a commercially available vitamin C supplement. This is the first report of the electrochemical properties of graphene nano-sheets produced using liquid-phase exfoliation, and it will serve as an important benchmark in the development of inexpensive graphene-based electrodes with high surface area and electro-catalytic activity.

1. Introduction

Carbon materials are widely used in electrochemistry due to their low cost, wide potential window and electro-catalytic activity for a range of redox reactions. Their applications in electro-catalysis depend very much on their microstructure and surface chemistry, and the various allotropes have very different electrochemical properties. The electrochemistry of ‘traditional’ sp²-hybridised carbon materials such as glassy carbon and highly ordered pyrolytic graphite has been thoroughly probed, but the last decade has seen an extraordinary amount of research into the electrochemical properties of carbon nanotubes (CNTs). Besides being quasi-one-dimensional electronic systems, they offer a high conductivity and surface area. Despite this, there remains a great deal of uncertainty regarding the precise mechanisms by which CNTs function as electrode materials. This arises from the use of different types of CNTs which have different electronic properties and contain various (and often unspecified) amounts of structural defects, metallic impurities and surface functionalities.

Graphene, the newest member of the carbon family, is attracting interest as an electrode material due to its two-dimensional nature. Strictly speaking, graphene is a carbon monolayer, but Geim and Novoselov have reported that structures composed of up to around ten graphitic sheets have electronic properties sufficiently different from bulk graphite to be classified as graphene. It should be pointed out that there is some disagreement in the literature regarding the point at which ‘graphene’ becomes ‘graphite’. A recent article by Pumera recommends that structures consisting of up to one hundred layers should be thought of as graphene. The first report concerning graphene electrochemistry was published in 2008 by Shang et al., who used as-grown arrays of vertically aligned graphene nano-sheets (GNSs) on silicon as dopamine sensors. The following year saw a number of papers reporting the modification of commercial glassy carbon electrodes (GCEs) with graphene nano-sheets produced by the chemical reduction of graphitic oxide. The modified electrodes have been used as sensors for various analytes, and as electro-catalysts for the oxygen reduction reaction. They have also been combined with the redox proteins glucose oxidase and cytochrome c in biosensors for glucose and nitric oxide, respectively. Graphene paper electrodes have been used in lithium batteries, and graphene oxide has been incorporated into a glucose biosensor. Recently, Qu et al. used nitrogen-doped graphene, produced using chemical vapour deposition, to promote oxygen reduction in fuel cells.

Ascorbic acid (AA) is used extensively in the food and drinks industry. The accurate determination of its concentration is of considerable importance, but the reliable sensing of AA using conventional carbon electrodes has been hindered by the large over-potentials required and electrode fouling by oxidation products. It is hoped that a reliable electroanalytical technique might offer greater selectivity, time efficiency and reproducibility than existing methods such as high-performance liquid chromatography, capillary zone electrophoresis and spectrophotometry. As a result, electrodes have been modified with carbon nanotubes and other agents such as cobalt phthalocyanine, ferrocene derivatives and ferrocene carboxylic acid for the detection of ascorbic acid. These efforts have met with various degrees of success.

In this paper, the development of a novel AA sensor based on pyrolysed photore sist films (PPFs) modified with graphene nano-sheets is reported. The latter are produced by the dispersion and exfoliation of graphite flakes. The fabrication process...
builds on our previous work describing the production of monolayer graphene. The sonication time has been extended in order to yield smaller flakes with greater electrochemical surface areas and higher electro-catalytic activity. The PPFs are made in-house and they provide an excellent electrical contact to the sheets. They serve as a cheap and disposable alternative to the glassy carbon electrodes used in previous reports. Furthermore, being themselves less electrochemically active than GCEs, they permit the de-convolution of kinetic data and facilitate a clearer understanding of the electrochemistry of the graphene nano-sheets. The ascorbic acid sensor is shown to exhibit impressive selectivity using tests involving a commercial vitamin C supplement. A key objective of the work is the evaluation of the fundamental electron transfer characteristics of graphene nano-sheets. This is done using standard redox probes, and new insights are presented regarding the reasons for the promotion of electron transfer by the sheets.

**Experimental**

**Chemicals and instruments**

Graphite powder (product number 332461), potassium chloride (99.0%), hexaammineruthenium(III) chloride (98%), potassium ferrocyanide (99.0%), potassium ferricyanide (99.0%) and ascorbic acid (99.0%) were purchased from Sigma-Aldrich. Rabex tablets were purchased from a local pharmacy. All solutions were prepared with water (resistivity 18.2 MΩ cm) from a Thermo Scientific millipore system. The 50 mM, pH 7 phosphate buffer solution used was prepared by dissolving monosodium dihydrogen phosphate dihydrate (NaH2PO4·2H2O) and disodium hydrogen phosphate dodecahydrate (Na2HPO4·12H2O) in water in the appropriate ratio. These two salts were purchased from Merck.

A Jeol 2100 TEM, operated at 200 kV, and a Zeiss Ultra SEM were used to obtain images of the graphene nano-sheets. Raman spectra were recorded using a Jobin-Yvon spectrometer set to an excitation wavelength of 633 nm, with a probe size of two microns. Five spectra were obtained for each sample in order to ensure uniformity. Thicknesses of pyrolysed photoresist films were determined using a Profilometer (Dektac 6M, Veeco Instruments), and conductivities were measured using a Jandel four-point probe. A Gamry 600 potentiostat was used to perform cyclic voltammetry and electrochemical impedance spectroscopy, along with a three-electrode configuration. IJ Cambria supplied platinum wire counter electrodes (product number CH1115) and Ag/AgCl reference electrodes (CH1111). Electrolytes were purged for twenty minutes using high-purity argon before experiments, and a blanket of argon was maintained over the solution during measurements. All electrochemical experiments were performed at room temperature (18 ± 3 °C).

**Formation of pyrolysed photoresist films**

Si/SiO2 wafers (1.5 × 1.5 cm) were cleaned using acetone and isopropanol, and then blown dry using compressed air. The photoresist (AZ nLOF 2070, MicroChemicals GmbH, Ulm, Germany) was spin-coated onto these substrates at 6000 rpm for 45 seconds, followed by baking (one minute at 100 °C). The samples were then annealed in a vacuum tube furnace under continuous flow of forming gas (90% argon and 10% hydrogen). The temperature was increased at a rate of 4 °C min⁻¹ to 1000 °C and held at this temperature for one hour. A Profilometer was used to show a decrease of thickness upon annealing by a factor of five, resulting in films of around 20 nm thickness, with conductivities around 1.6 × 10⁴ S m⁻¹, as measured using a four-point probe.

**Formation of graphene nano-sheets and electrode modification procedure**

Graphite flakes were dispersed in dimethylformamide (3.3 mg cm⁻³) and placed in a sonic bath for 72 hours to yield graphene nano-sheets. Surviving flakes were removed by centrifuging at 750 rpm for 45 minutes, followed by decanting the supernatant. When required, the latter was dropped (15 μL) directly onto photoresist films and the solvent was allowed to dry. The resulting GNS films were clearly visible to the naked eye. The GNS-modified PPFs were incorporated into the electrochemical cell by placing the substrates in an electrode designed in-house (see ESI†), resulting in a disc electrode (radius 1.5 mm). The PPF and PPF/GNS surfaces were activated before measurements by cycling in background electrolyte until reproducible scans were obtained. Three or four cycles were usually found to be sufficient to achieve this. The potential ranges over which these preliminary cycles were carried out were chosen to match those of whatever measurement was to be subsequently performed.

**Results and discussion**

The morphology of the GNSs was investigated using transmission electron microscopy and scanning electron microscopy. Fig. 1(a) shows a typical TEM image of graphene sheets drop-coated onto a holey carbon grid. The nano-sheets are mostly sub-micron in lateral size, of various thicknesses, and in places appear to be stacked on top of each other. A statistical analysis of one hundred arbitrary flakes revealed that ~90% had five layers or fewer (see ESI†). Fig. 1(b) shows a SEM image of a drop-coated GNS film on a PPF electrode surface. Some of the nano-sheets have edges which are wrinkled up away from the underlying electrode, like paint peeling off a wall. Images of the bulk graphite source from which the sheets were produced can be found in previous work, showing flakes at least 150 μm across, so it is clear that the prolonged sonication results in a considerable decrease in flake size. The GNSs were further characterised using Raman spectroscopy, and Fig. 1(c) shows a spectrum averaged over five spots on a GNS assembly formed by drop-coating onto a Si/SiO2 wafer. Included in the figure is a spectrum recorded for the as-received graphite. The two spectra were normalized to the intensity of the G peak (1582 cm⁻¹). The larger D band (1330 cm⁻¹) observed for the GNSs confirms the smaller domain size, and the broader 2D peak (2660 cm⁻¹) indicates the formation of thin graphic sheet's of various thicknesses, as opposed to bulk graphite.

The diminished flake size means that the GNS material has a larger density of edge-plane sites and defects, which are known to promote electron transfer at carbon electrodes.

The presence of graphene nano-sheets on the pyrolysed photoresist film surfaces was found to increase the electro-chemically active surface area. The hexaammineruthenium(III) chloride redox probe was used to quantify this increase. This probe was selected for this purpose because its reaction kinetics
are determined by the Fermi level density of states of the electrode material, rather than any functionalities, defects or impurities which may be present. This means that it shows reversible electrochemistry at virtually all graphitic surfaces, enabling the legitimate comparison of peak currents without the need to consider differences in reaction kinetics. Fig. 2 shows a typical comparison between bare and modified electrodes. The larger peak current for the PPF/GNS electrode is attributed to a greater electrochemically active surface area. Values for these areas were calculated using the equation

\[ i_{pc} = 3.01 \times 10^7 \times n^{3/2} AC^n \sqrt{\alpha Dv}. \]  

in which \( i_{pc} \) is the cathodic peak current, \( n \) is the number of electrons involved in the redox process (\( n = 1 \) for the \([\text{Ru(NH}_3)_6]^{3+}/2+\) couple), \( A \) is the electrochemical surface area, \( C^n \) is the bulk concentration of the redox probe, \( \alpha \) is the transfer coefficient (taken to be 0.5), \( v \) is the voltammetric sweep rate and \( D \) is the diffusion coefficient of the electro-active species, which in this case has the value of \( 6.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}. \) Based on peak current values of 23.9 and 35.7 \( \mu \text{A} \) obtained at a scan rate of 200 mV s\(^{-1}\), the electrochemical surface area was found to increase from 0.10 to 0.15 cm\(^2\) in the presence of the sheets. It is noted that, due to roughness, both of these values are larger than the geometric area of the electrodes (0.07 cm\(^2\)).

The standard, quasi-reversible ferro/ferricyanide redox probe was used to elucidate the electron transfer properties of the graphene nano-sheets. Fig. 3(a) compares cyclic voltammograms obtained using bare and GNS-modified pyrolysed photolithography films. It is clear that, for this probe, the electron transfer at the bare PPF is very sluggish, with only slight waves corresponding to the redox process. This is an important point. Virtually all other studies reporting GNS electrochemistry involve the modification of commercial glassy carbon electrodes. We point out that the latter are themselves endowed with excellent electron transfer properties, and therefore the kinetic data obtained can be somewhat convoluted. We suggest that it is preferable to use relatively inert underlying electrode surfaces such as the PPFs described here. Returning to Fig. 3(a), in the presence of GNSs, a well-defined response is obtained, suggesting a dramatic enhancement in the rate of electron transfer. Fig. 3(b) shows that, for the PPF/GNS electrode, the anodic peak current is proportional to the square root of the sweep rate over the range 5 to 200 mV s\(^{-1}\) (correlation coefficient 0.999). This behaviour is characteristic of diffusion-controlled electrochemical reactions. In order to provide a quantitative insight into the electron transfer properties of the graphene nano-sheets, the standard electrochemical rate constant was calculated for the ferro/ferricyanide test reaction. This was done using the Nicholson method, which yields values for this parameter based on voltammetric peak separations. The separation at a scan rate of 100 mV s\(^{-1}\) was found to be 120 mV, which yielded a value of 3.5 \( \times 10^{-3} \text{ cm}^2 \text{ s}^{-1} \) for the rate constant. A more detailed description of the application of this method can be found in previous work. It is noted that this value is similar to that reported by Pacios et al. using bamboo-like carbon nanotube composite electrodes, employing the same approach.
The enhanced electron transfer properties of the GNS-modified electrodes were also clear from electrochemical impedance spectroscopy data. Fig. 4 shows a comparison between Nyquist plots obtained using a PPF electrode in the absence and in the presence of graphene nano-sheets. In such plots, the resistance to charge transfer is indicated by the size of the semicircular region. It can be seen that the response of the bare electrode is dominated by this feature, suggesting that the reaction kinetics are under the control of electron transfer at all frequencies. However, the modified electrode gives rise to a far smaller charge transfer resistance, with a considerable linear region indicating diffusion control at moderate frequencies. The impressive properties of the GNS electrodes are attributed to their high density of edge-plane sites and defects, which have been established as key participants in the promotion of electron transfer at carbon electrodes.

Despite the thinness of the flakes (see ESI†), it is not envisaged that the excellent electrochemical activity of this material is due to its much-lauded electronic properties. The key to its performance is the small lateral size of the flakes created by prolonged sonoecation, and the resulting large proportion of edge regions. The impressive properties of the GNS electrodes are attributed to their high density of edge-plane sites and defects, which have been established as key participants in the promotion of electron transfer at carbon electrodes.30–35

Using a fixed sweep rate of 100 mV s$^{-1}$, the variation of the anodic peak current, $i_{pa}$, with ascorbic acid concentration was investigated. Fig. 6(a) shows the increase of $i_{pa}$ with successive additions of AA, and the resulting calibration plot in Fig. 6(b) is a straight line (correlation coefficient 0.997) for concentrations between 0.4 and 6.0 mM, given by the equation:

$$i_{pa} (\mu A) = 18.5 \times C_{AA} (mM) - 7.37$$

in which $C_{AA}$ is the concentration of ascorbic acid. The limit of detection (based on 3$\sigma$) was found to be 0.12 mM.

Cyclic voltammetry was used to investigate the application of graphene nano-sheets in electrochemical sensors for ascorbic acid. Fig. 5 shows the effect of GNSs on the response of a pyrolysed photoresist film electrode to this analyte. While the bare electrode shows only a wave corresponding to AA oxidation, the PPF/GNS electrode gives a well-defined peak around +0.37 V (vs. Ag/AgCl). No significant decrease in peak current was observed on twenty successive measurements (see ESI†), suggesting that this material is not subject to deleterious adsorption processes such as those reported for ascorbic acid on glassy carbon electrodes.41
The sample. contributions from glucose or other interfering species present in due entirely to ascorbic acid, and there are no significant mined concentration of 2.61 mM. This confirms that the signal is spike gives 2.64 mM, which is very close to the originally deter-
corresponds to a concentration of 4.67 mM. Subtraction of the 
produced by the dispersion and exfoliation of graphite have been 
made in a 20 cm³ sample. This means that the tablet 
was 31% ascorbic acid, which is in reasonable agreement with the 
report. This material has been shown to consist of graphene 
and defects. Standard redox probes have been utilised to 
demonstrate the large electrochemical surface area and electro-
catalytic activity of the graphene nano-sheets. The unique 
exfoliation process is preferable to the more common chemical 
reduction of graphitic oxide, in that it permits the definitive 
identification of edge-planes as the promoters of electron trans-
fer, an issue of continuing debate in the field of nano-carbon 
electrodes. A novel ascorbic acid (vitamin C) sensor based on 
pyrolysed photoresist films modified with GNSs has been 
demonstrated. This system shows no evidence of deleterious 
adsorption processes, and its accuracy and selectivity have been 
verified using a commercially available vitamin C supplement. 
These findings constitute a significant step in the development of 
a new class of nanostructured electrodes for sensors, fuel cells 
and energy conversion.

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