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Chemically modified graphene and nitrogen-doped graphene: Electrochemical characterisation and sensing applications

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ABSTRACT

Functionalised graphene (G) and nitrogen doped graphene (NG) nanomaterials are excellent candidates for electrocatalytic sensing of biomolecules and for developing biosensors, due to their unique physicochemical and electronic properties. Electrochemical characterisation and comparison of basic or acidic functionalised G and NG has been carried out, as well as of composite materials based on NG with the conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) and the redox polymer poly(neutral red) by cyclic voltammetry and electrochemical impedance spectroscopy. Electroactive areas and heterogeneous electron transfer constant, of the GCE modified with the graphene derivatives have been evaluated, in order to choose the best material for electrode modification. The NG modified GCE enabled excellent electrocatalytic regeneration of the enzyme cofactors β-nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), underlining the applicability of NG for the development of new sensitive biosensors.

1. Introduction

Direct electron transfer at bioelectronic interfaces is one of the key questions in developing biosensor technology, as well as significantly enriching biofuel cell development. The redox centres of most enzymes are embedded deep inside the enzyme, and facilitating efficient electron transfer to the electrode surface is a challenging task [1]. Several methods have been adopted in order to establish electrical communication between redox enzymes and electrodes, first by using carbon nanotube, metal nanoparticle or graphene modified electrodes as sensor substrates, and secondly by employing electron transfer mediators, tethering of redox relay units to enzymes, or reconstitution of the apo-enzyme on relay cofactor units associated with electrodes [1–6].

During the past few decades, carbon-based nanomaterials have been widely used in electrochemical sensors due to their excellent catalytic activity, superior conductivity, large surface area, ease of functionalisation and biocompatibility [7]. They include the conventional carbon black, graphite, carbon nanotubes, fullerenes, up to the latest innovation of 2D graphene nano sheets. Graphene has been an attractive material due to its excellent conductivity, feasibility for microfabrication, high surface area, mechanical strength, optical transparency and biocompatibility [8,9]. Graphene consists of sp^2 hybridised carbon atoms, in which the valence and the conduction band overlap at the Brillouin zone, making pure graphene a zero band gap semiconductor, which limits its application potential due to its chemical inertness [10,11].

Doping with heteroatoms such as boron or nitrogen is an excellent method to open up the band gap and provide pathways for efficient electron transfer processes, transforming graphene into a p- or n-type semiconductor, a promising material in electrochemical biosensing, in supercapacitors and in fuel cells [10–13]. Up until now, such heteroatom doping was found to be successful in carbon nanotubes [14,15]. The introduction of a band gap can generate remarkable properties in graphene, analogous to CNTs. The one-dimensional nature of CNTs makes it difficult to controllably assemble CNTs whereas the 2D nature of graphene makes it suitable for microfabrication. Another important advantage of graphene over CNTs is the absence of metallic impurities [16,17]. Theoretical studies on adsorbate or substitutional B, N or O doped graphene has proved that substitutional doping, when heteroatoms are incorporated into the honeycomb structure of graphene, is more effective than the adsorption of heteroatoms on the graphene surface [12]. Substitution with an electron-rich heteroatom such as N, P etc. will result in n-type characteristics whereas an electron deficient atom like B induces a p-type characteristics, both of which cause an increase in free charge carriers in the graphene framework, thereby enhancing conductivity. Hence tuning the band gap

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by chemical doping enhances the charge carrier concentration [18] and leads to promising applications in semiconductor electronics such as field effect transistors [11], and as electrocatalyst in the oxygen-reduction reaction (ORR) in fuel cells and in sensors [19].

The present study focuses on the electrochemical characterisation of functionalised graphene (G) and N-doped graphene (NG) and their application in sensing and biosensing. The N-doping of graphene has been done by thermal annealing in the presence of ammonia and the nitrogen atom in the graphene framework can exist in "graphitic", pyridinic or pyrrolic forms, each of which can provide different characteristics to the graphene framework [19,20]. Pure and N-graphene were compared with their derivatives, which were acidic (HNO_3) or basic (KOH) functionalised G and NG [21,22]. Composite materials based on NG with the conducting polymer poly(3,4-ethylenedioxythiphene) (PEDOT) and the redox polymer poly(neutral red) (PNR) were also synthesised. To fully exploit the electrochemical properties of the different types of graphene and composite graphene, electrochemical characterisation was performed at graphene modified GCE by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) without and with the standard electroactive species $K_4[Fe(CN)_6]$. To our knowledge, the electrochemistry of NG and its acidic and/or basic functionalised analogues and polymer composites with NG have not yet been thoroughly investigated, so that the present work brings a deeper knowledge and comparison of their electrochemical characteristics. The graphene material with the best electrochemical properties has been applied to the detection of important cofactors present in oxidase and dehydrogenase based enzymes, i.e. β-nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), the regeneration of which is the crucial step during an enzyme reaction, and thence for biosensor performance.

2. Experimental

2.1. Reagents and buffer electrolyte solutions

All reagents were of analytical grade and were used without further purification. Graphene and N-doped graphene, prepared by thermal reduction of graphite oxide, were characterised in [23]. Graphite, chitosan (low molecular weight), 2,3-dihydrothieno[3,4-*b*]-1,4-dioxin (EDOT), glutaraldehyde, neutral red (NR) 65% dye content, nicotinamide dinucleotide, monobasic and dibasic potassium phosphate, sodium chloride and sodium poly(styrene sulfonate) (NaPSS) were from Sigma-Aldrich, Germany. Flavine adenine dinucleotide, potassium hexacyanoferrate(II) trihydrate, potassium chloride, monobasic sodium phosphate and were obtained from Fluka, Switzerland.

For electrochemical experiments, the supporting electrolytes were 0.1 M KCl or sodium phosphate buffer saline (NaPBS) (0.1 M phosphate buffer + 0.05 M NaCl, pH = 7.0). Polymerisation of NR was carried out in 0.025 M potassium phosphate buffer solution plus 0.1 M KNO_3 (pH 5.5) containing 1 mM NR and of EDOT in 0.1 M NaPSS containing 10 mM EDOT.

Millipore Milli-Q nanopure water (resistivity $\geq$18 MΩ cm) was used for the preparation of all solutions. All experiments were performed at room temperature (25 ± 1 °C).

2.2. Instrumentation

Electrochemical experiments were performed in a three electrode cell, containing a glassy carbon electrode (GCE) (area 0.237 cm^2) as working electrode, a Pt wire counter electrode and an Ag/AgCl (3.0 M KCl) reference electrode, using a potentiostat/galvanostat μ-Autolab system (Metrohm-Autolab, Netherlands).

Electrochemical impedance spectroscopy (EIS) experiments were carried out with a potentiostat/galvanostat/ZRA, (Gamry Instruments, Reference 600). An rms perturbation of 10 mV was applied over the frequency range 100 kHz to 0.1 Hz, with 10 frequency values per frequency decade.

The pH measurements were carried out with a CRISON 2001 micro pH-meter (Crison Instruments SA, Barcelona, Spain) at room temperature.

2.3. Functionalisation of graphene and N-graphene and preparation of modified electrodes

Graphene (G) and nitrogen doped G (NG) were used as received or treated either in 3 M HNO_3 or in 7 M KOH. For the acidic treatment, the graphene or graphite (Gr) powder was stirred during for 12 h, while for the treatment with base, stirring was during 4 h followed by another 20 h static soaking in ambient conditions. The functionalised particles were then washed and filtered with Milli-Q water until the solution become neutral. The material obtained was dried at ≈60 °C overnight. In this way we obtained HNO_3_G/NG/Gr and KOH_G/NG. Due to the fact that HNO_3 treatment of NG was detrimental, the dispersion not being homogeneous and the modified electrodes being very unstable, this material was not used further in this study.

Both functionalised and un-functionalised G and NG were dispersed in 1% (w/v) chitosan dissolved in 1% (v/v) acetic acid, to form a 0.1% dispersion. The solution was sonicated for 1 h and vortexed before 20 μl was drop cast on the GCE. The modified electrodes were left overnight to dry.

2.4. Preparation of PEDOT/NG and PNR/NG modified electrodes

Four types of composite of NG with conducting polymer PEDOT and the redox polymer PNR were prepared: NG/PEDOT, PEDOT/NG, NG/PNR and PNR/NG. In the first and third case, the GCE was first drop cast with NG followed by electropolymerisation of EDOT and NR, whereas in the second and fourth, electropolymerisation of EDOT/NR was done prior to the drop casting of NG.

Both monomers were electropolymerised by cyclic voltammetry in the solutions described in Section 2.1, at a scan rate of 50 mV s^{-1}, between −1.0 and +1.0 V vs. Ag/AgCl for 15 cycles, for NR and from −0.6 V to 1.2 V vs. Ag/AgCl for 20 cycles for EDOT.

3. Results and discussion

Elemental, structural and surface morphological characterisation of the graphene and N-doped graphene has been carried out previously [23], one of the main conclusions being that more defects are present on nitrogen-doped graphene.

The electrochemical characterisation of the G and NG modified electrodes and their functionalised derivatives was undertaken in order to evaluate potential windows, electroactive areas and apparent heterogeneous rate constant (k_0). EIS measurements allowed confirmation of results obtained by CV and, furthermore, to explore surface and bulk characteristics of the graphene-modified electrodes. This permitted choosing the best graphene materials for sensing/biosensing application. Their electrocatalytic properties for the determination of the very important enzyme cofactors NADH and FAD was evaluated.