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Functionalized polypyrrole/sulfonated graphene nanocomposites: Improved biosensing platforms through aryl diazonium electrochemistry

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ABSTRACT

A novel electrochemical approach aimed at developing biosensing platforms based on polypyrrole/sulfonated graphene nanocomposites is reported. Specifically, nanocomposite layers are deposited onto platinum electrodes through the electrochemical polymerization of pyrrole monomer in the presence of reduced graphene oxide bearing phenylsulfonyl groups. Thus, the functionalized graphene nanofiller acts as dopant and balances the positive charges on the polymer chains, leading to an enhancement of the polymer's electrical conductivity and concomitantly increasing the electrode surface area. The polypyrrole/graphene nanocomposite films are further modified with carboxyphenyl groups via electrochemical reduction of 4-carboxyphenyl diazonium tetrafluoroborate. Grafting carboxyphenyl functionalities serves a dual purpose: it permits the covalent immobilization of glucose oxidase via carbodiimide chemistry and also forms an electrode blocking layer which hinders the oxidation of interfering substances. The feasibility of this strategy is demonstrated by the preparation of a glucose biosensor which exhibited an improved performance: wide linear range (0.02–12 mM), good sensitivity (0.56 μA mM^{-1} cm^{-2}) and adequate selectivity towards common interferents including ascorbic acid, paracetamol, uric acid, and cysteine.

1. Introduction

Graphene is a two-dimensional material with outstanding physical, chemical, and electrical properties that make it very attractive for developing high performance biosensors for a variety of analytes [1,2]. Its excellent electrical conductivity, high specific surface area, exceptional biocompatibility, easy functionalization, low environmental impact, and low cost are major factors contributing towards graphene and its related composites use in electrochemical biosensors technology [3]. Graphene has been competing with carbon nanotubes by showing excellent performance for the electrochemical detection of small biomolecules [4]. However, pristine graphene possesses some inherent disadvantages such as easy aggregation, poor solubility and/or processability that can manifest as impediments in biosensor related applications [5]. Therefore, in order to improve the performance or to fulfill the specific requirements of different kinds of biosensors, graphene can be functionalized through various covalent and non-covalent methods [6,7]. Graphene can also be combined with other functional materials such as polymers, to obtain more sensitive, selective, and more reproducible devices for electroanalysis [8]. In this regard the incorporation of graphene as nanofiller in different polymer matrices has been shown to substantially improve material characteristics such as increased surface area and higher number of analytical recognition sites, redox behaviour and biocompatibility [9,10]. The enhancement of electron transfer kinetics and high sensitivity results from the good conductivity and large surface area of graphene. Combining polymers with graphene brings also additional advantages such as improving the solubility/processability of graphene in both water and organic solvents while preventing the stacking of graphene sheets [11]. The highest percentage among these polymers is accounted by conducting polymers [12], with polypyrrole (PPY) playing a leading role because of its stability, facile synthesis, easy processability and environmental stability [13–21].

It is well known that in the last decades polypyrrole was extensively

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used in amperometric biosensors as a matrix for glucose oxidase (GOx) immobilization, the enzyme being incorporated during polymer deposition by electrochemical oxidation of the monomer [22,23]. Electrochemical polymerization provides a one-step procedure and offers precise control of the thickness and the structure of the resulting film [24]. In addition, the overoxidation of PPY films is a general procedure adopted for reducing its electroactivity in the potential window used for analyte detection but can also lead to polymer degradation [25]. The incorporation of graphene can surpass this drawback by increasing the active surface area available for the immobilization of biomolecules and facilitating the electronic transfer between the analyte and the electrode [26]. To date, electrochemically synthesized graphene –conductive polymer composites have attracted considerable attention as electrode materials. Recently, the use of sulfonated graphene sheets as a dopants for conducting polymers was presented as novel route to prepare graphene-polypyrrole nanocomposites for supercapacitors [27]. All further reports regarding polypyrrole/sulfonated graphene [13] or polyaniline/sulfonated graphene nanocomposites [28] dealt with assessing the performance of such materials in supercapacitor related applications. Many other studies have been dedicated to other types of conductive polymers/graphene nanocomposites, but only few were related to the use of these materials for electrochemical biosensors [29–33].

In this work we propose a new approach for glucose biosensor fabrication starting from sulfonated graphene/polypyrrole nanocomposite films. Nanocomposite layers are deposited onto platinum electrodes through the electrochemical polymerization of pyrrole monomer in the presence of reduced graphene oxide bearing phenylsulfonyl functionalities. Functionalized graphene acts as dopant and balances the positive charges on the polymer chains, thus enhancing the electrical conductivity of the polymer and increasing the surface of the electrode. In order to facilitate the covalent linking of GOx, which is expected to increase the biosensor lifetime, the nanocomposite electrodes are subsequently modified with carboxyphenyl groups through the electrochemical reduction of the corresponding diazonium salt, a functionalization method reported by our group for conducting polymers [34]. Furthermore, this procedure should also increase biosensor selectivity, as we have previously demonstrated that polyaryl films grafted via the electrochemical reduction of aryl diazonium salts act as blocking layers which hinder the oxidation of interfering substances [35].

2. Experimental section

2.1. Chemicals and instrumentation

Graphene oxide (2 mg mL^{-1} aqueous dispersion) was purchased from Aldrich. Anhydrous acetonitrile (99.8%, noted MeCN), tetra-*n*-butylammonium tetrafluoroborate (99%, noted $TBA^+BF_4^-$), *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) were obtained from Aldrich and were used as received. Glucose oxidase (type X-S from Aspergillus niger, 250,000 U g^{-1}) was purchased from Sigma. Pyrrole acquired from Merck was distilled and stored at −20 °C under an inert atmosphere before use. Sodium sulfanilate, D-(+)-glucose, ascorbic acid, uric acid, and paracetamol were used as received (Sigma- Aldrich). All other chemicals were of analytical grade.

4-Carboxyphenyl diazonium tetrafluoroborate was prepared by diazotization of 4-aminobenzoic acid with sodium nitrite in fluoroboric acid medium [36,37].

2.1.1. Synthesis of 4-carboxyphenyldiazonium tetrafluoroborate

A solution of 1.73 g (25 mmol) sodium nitrite in 2 mL water was added during approx. 30 min to a stirred suspension of 3.42 g (25 mmol) 4-aminobenzoic acid in 7 mL of 48% fluoroboric acid. The suspension was maintained at −20 °C in an ice-salt bath during the addition, and afterwards the resulting mixture was kept at the same temperature for another 30 min. The product was vacuum-filtered on a sintered glass crucible and washed successively with small quantities of cooled 48% fluoroboric acid, 96% ethanol and diethyl ether. The diazonium tetrafluoroborate was dried at room temperature in a vacuum desiccator (5 mmHg) for 1 h and stored at −20 °C.

4-Carboxyphenyldiazonium tetrafluoroborate (^{1}H NMR, 300 MHz, CD_3CN, ppm) δ: 8.41 (d, 2H, J = 9.0 Hz), H3 and H5; 8.60 (d, 2H, J = 9.0 Hz), H2 and H6.

From the integrals of residual signals at 7.5 and 8.1 ppm corresponding to unreacted 4-aminobenzoic acid, the purity of the diazonium tetrafluoroborate was found to be 95%.

2.1.2. Synthesis of aminomethylferrocene hydrochloride

Aminomethylferrocene hydrochloride was synthesized by the reductive amination of ferrocene carboxaldehyde with sodium cyanoborohydride in the presence of ammonium acetate followed by treatment with methanolic HCl, as described in Ref. [38].

Aminomethylferrocene hydrochloride (^{1}H NMR, 300 MHz, DMSO-*d6*, ppm) *δ*: 3.75 (s, 1H) and 3.81 (s, 1H), $-CH_2-NH_3^+$; 4.19–4.23 (m, 7H), ortho H of Cp ring and H from unsubstituted Cp ring; 4.37 (s, 1H) and 4.42 (s, 1H), meta H of Cp ring; 8.29 (s, br, 3H), $-NH_3^+$.

Electrochemical experiments were performed using an Autolab 128N (Metrohm Autolab B.V.) potentiostat and a conventional three-electrode system with a platinum-modified electrode (2 mm diam. disk, Metrohm) as the working electrode, a platinum wire as the counter electrode, and Hg/Hg_2Cl_2 (3 M KCl) as reference electrode (Metrohm). For nonaqueous solutions, an Ag/Ag^+ (0.01 M $AgNO_3$ and 0.1 M $TBA^+BF_4^-$ in MeCN) reference electrode was employed. Unless otherwise stated, all experiments were carried out at room temperature in phosphate buffer solution (0.1 M, pH 7.4).

Scanning electron microscopy (SEM) examination was performed using a Quanta Inspect F SEM (FEI Co.) equipped with a field emission gun.

Raman spectra were recorded in the range 3500–500 cm^{-1} using a dispersive Raman microscope DXR (Thermo Fisher Scientific) employing a 532 nm laser with a power of 10 mW. X-ray photoelectron spectra were acquired on a K-Alpha spectrometer (Thermo Scientific) operating with monochromatic Al Kα X-rays (1486.6 eV). Proton NMR spectra were recorded on a Fourier 300 spectrometer (Bruker).

2.2. Graphene functionalization

For the functionalization of graphene we modified slightly the three-step procedure described by Si and Samulski [39]. Briefly, 20 mL of 2 mg mL^{-1} graphene oxide (GO) aqueous dispersion were reduced with 400 mg sodium borohydride at 80 °C for 1 h to remove the majority of oxygen functionalities. The pre-reduced graphene oxide was dispersed in 40 mL water and reacted in the second step with the aryl diazonium salt prepared from 40 mg sodium sulfanilate at 5 °C for 4 h. Finally, the sulfonated graphene was redispersed in 40 mL water and reduced with 1 mL hydrazine monohydrate at 100 °C for 20 h, in order to remove the remaining oxygen functionalities.

2.3. Preparation of the modified electrodes

Prior to modification, the Pt electrode surface was polished successively with 1 μm diamond slurry and 0.05 μm alumina slurry on a microcloth pad. After polishing, electrodes were thoroughly rinsed with water and sonicated for 5 min in water. Functionalized graphene (RGO-$PhSO_3^-$) was dispersed in bidistilled water by sonication for 1 h, at a concentration of 0.2 mg mL^{-1}. Pyrrole was then added to the stable dispersion up to a final concentration of 0.2 M and the electropolymerization onto Pt electrodes was performed galvanostatically at current densities of 0.1, 0.2, or 0.4 mA cm^{-2} (the transferred charge was 0.24C cm^{-2} for each current density). Afterwards, the composite