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Design of a reduced-graphene-oxide composite electrode from an electropolymerizable graphene aqueous dispersion using a cyclodextrin-pyrrole monomer. Application to dopamine biosensing

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ABSTRACT

A new cyclodextrin-modified pyrrole monomer (Py-CD) was synthesized and used both as an aqueous surfactant for the stabilization of aqueous reduced graphene oxide (RGO) dispersion and as an electropolymerizable unit to generate a RGO-polypyrrole composite electrode. This nanocomposite was used in combination with amphiphilic pyrrole derivative for the entrapment of tyrosinase, an enzyme catalyzing the oxidation of phenols and diphenols. Compared to glassy carbon electrodes, these nanostructured electrodes exhibit enhanced electroactive surface area and electrochemical properties and achieve higher performances towards catechol and dopamine biosensing.

1. Introduction

Graphene, a two-dimensional sheet of carbon atoms, presents extraordinary properties such as a very large surface area, high mechanical strength, high elasticity and thermal conductivity. The electrochemical properties of graphene, graphene oxide (GO) and reduced graphene oxide (RGO) are widely studied and represent excellent candidates for carbon-based electrode materials in a wide range of applications [1–4]. Similarly to carbon nanotubes, graphene can be functionalized by covalent or non-covalent methods, targeting the sp^2 extended carbon network, giving access to novel nano-objects with versatile physico-chemical properties [5–7]. In particular, non-covalent techniques have the great advantage of not damaging the pi-extended network of benzene rings, thus preserving the graphene conductivity and therefore its use as electrode material.

Because of these interesting properties, graphene has been used as a transducer in bio-Field-Effect-Transistors, electrochemical biosensors, impedance biosensors, electrochemiluminescence, and fluorescence biosensors, as well as biomolecular labels. The applications of graphene in (bio)sensing are based on electrodes modified with graphene powder or graphene-composite electrodes. Different methods have been developed to build graphene-based electrodes: drop-coating, scotch tape transfer, vacuum filtration, CVD growth, electrochemically-reduced graphene oxide electrodes. It is noteworthy that, while CVD-grown graphene and soft graphene exfoliation can give access to mono- to few-layer graphene, reduction processes of graphene oxide represent an easy and cheap access to gram-scale samples, despite a higher amount of defects or stacked graphene sheets.

A challenging aspect in this area is the elaboration of hybrid electrodes based on the combination of graphene and conductive polymers leading to the design of novel nanocomposites with (bio)sensing properties.

Rapid, localized and sensitive detection of neurotransmitters such as dopamine is of particular interests in the study of nervous system functions and disorders. Among dopamine sensing methods, electrochemical biosensing possesses many advantages such a

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simplicity, low costs, use of miniaturized and portable equipment [8]. In particular, the use of immobilized enzymes takes advantages of high specificity and high catalytic turnovers of enzymes for the design of highly sensitive and selective biosensors of phenolic and diphenolic compounds such as catechol or dopamine [9]. Amperometric biosensors based on tyrosinase (or polyphenol oxidase) enzyme that catalyzes the oxidation of phenol derivatives in the presence of oxygen, thus constitute highly selective devices for ortho-diphenolic compounds. The main challenge in tyrosinase-based biosensor fabrication is the efficient immobilization of a high amount of enzyme per surface unit, accompanied with excellent electron transfer properties of the electrode surface towards the reduction of enzymatically generated quinoid compounds. Among different strategies of immobilization, conjugated polymers have proven to provide excellent microenvironments for enzymes and biocompatibility towards in vivo applications [10–14].

We and others have recently demonstrated that three-dimensional bio-functionalized architectures enhance biosensing performances [15]. In particular, the use of carbon nanotube supports provides a combination of high electroactive area, high stability and high conductivity [16,17].

In this work, we report an original approach for designing graphene-based composite electrode. This method is based on the non-covalent modification of RGO using a new synthesized pyrrole monomer bearing a β-cyclodextrin (β-CD) moiety. Taking advantage of the well-known use of β-CD as an efficient aqueous dispersant of graphene [18], this β-CD-pyrrole derivative acts as a surfactant of graphene in water and as an electropolymerizable unit, both aspects being beneficial for the functionalized graphene electrodeposition. Functionalized graphene sheets and composite electrodes were characterized by imaging, spectroscopic and electrochemical techniques. Moreover, these graphene-based composite electrodes were used in combination with an amphiphilic pyrrole derivative for the immobilization of tyrosinase and the design of a catechol and dopamine biosensor.

2. Experimental

2.1. Synthesis

[12-(pyrrol-1-yl) dodecyl] triethylammonium tetrafluoroborate (NEt_4^+-pyrrole)[19] and N-succinimide-11-pyrrolyl-1-undecyl carboxylic acid ester (NHS-pyrrole) [20] were synthesized according to previously described procedures.

Synthesis of: β-cyclodextrin-11-pyrrolyl-1-undecyl carboxylic acid amide (β-CD-pyrrole): The NHS-pyrrole (90 mg, 0.26 mmol) was dissolved in 5 mL of DMF. 1-adamantylamine (370 mg, 0.32 mmol) and an excess of tetraethylamine (150 mg, 1.5 mmol) were added and the resulting mixture was stirred overnight at 80 °C. The crude solution was evaporated to dryness and the residue was washed with water and Et_2O. 330 mg (0.24 mmol) of a white powder was obtained in 92% yield.

^{1}H NMR: δH/ppm (400 MHz, DMSO): 1.3-1.5 (m, 16H), 1.66 (t, j = 6.4 Hz, 2H), 2.07 (t, J = 7.2 Hz, 2H), 3.30 (m, 12H), 3.55(m, 28H),), 3.63 (t, j = 6.4 Hz, 2H), 4.42 (m, 7H), 4.82 (m, 7H), 5.64-5.70 (m, 14H), 5.92 (s, 2H), 1.44 (m, 2H), 6.71 (s, 2H), 7.55 (m, 1H)

MS (ESI+): 1367.5$^+$ (M^+)

2.2. Methods and Instrumentation

All reagents and chemicals were purchased from Aldrich and used as received. Tyrosinase (T3824-50kU) was purchased from Sigma. Supporting electrolytes were 0.1 M phosphate buffer (pH 6.5) and 0.1 M $LiClO_4$.

Reduced graphene oxide (RGO) was prepared by reducing graphene oxide according to previously-described procedures using ascorbic acid as a reducing agent [18,21].

For preparing RGO/β-CD-pyrrole solution, 1 mg mL^{-1} RGO and 1 mM β-CD-pyrrole were sonicated together for 3 h in water to give a stable black solution. For preparing NEt_4^+-pyrrole/tyrosinase solution, 10 mg mL^{-1} enzyme was dissolved in 3 mM NEt_4^+-pyrrole aqueous solution.

The electrochemical studies were performed with a conventional three-electrode system using $Ag/AgNO_3$ reference in MeCN or a saturated calomel electrode (SCE) in water. A Pt wire electrode was used as counter electrode. The working electrodes were glassy carbon (3 mm diameter), polished with 2 μm diamond paste followed by rinsing with distilled water and ethanol. Electrochemical experiments were conducted on an Autolab PGstat100 potentiostat.

2.3. Elaboration of electrodes and characterization

The GCEs were prepared by drop casting 5 μL of RGO/β-CD-pyrrole aqueous suspension, dried under vacuum and then electropolymerized by controlled potential electrolysis (0.85 V, 5 min) in 0.1 M $LiClO_4$/MeCN. A defined amount of NEt_4^+-pyrrole/tyrosinase aqueous solution (containing 0.3 mg of enzyme) with and without RGO/β-CD-pyrrole was spread on the electrode surface, dried under vacuum and then electropolymerized by controlled potential electrolysis for 20 min at 0.85 V in 0.1 M $LiClO_4/H_2O$.

The FTIR spectra were recorded with a Thermo Scientific Nicolet iS10 in the wave number range from 650 to 4000 cm^{-1}. TEM images were recorded using a Philips CM200 microscope operating at an accelerating voltage of 200 kV, equipped with a TemCam F216 TVIPS camera.

3. Results and discussions

Aqueous solution of β-CD-pyrrole/RGO was obtained by sonication of a solution containing 1 mg mL^{-1} RGO and 1 mM of β-CD-pyrrole. IR spectrum of the β-CD-pyrrole/RGO powder indicates the modification of RGO with β-CD-pyrrole. The presence of bands at 2928 cm^{-1} (C-H stretch in alkanes), 1201 cm^{-1}, 1148 cm^{-1}, 1075 cm^{-1}, 1020 cm^{-1} (C-O stretch in alcohols and ethers) attributed to β-CD molecules confirmed that the RGO sheets were successfully functionalized with β-CD-pyrrole (Fig. 1B).

It should be noted that the attempts to disperse RGO sheets in the absence of β-CD-pyrrole, have provided aggregates like “black flakes”. In contrast, the presence of β-CD-pyrrole greatly facilitates the RGO sheet dispersion, this dispersive effect being also observed with the naked eye (inset, Fig. 1C). The TEM images show the excellent individualization of graphene sheets, thanks to the dispersing properties of β-CD-pyrrole. The β-CD-pyrrole-modified RGO sample exhibits highly exfoliated RGO sheets with thin wrinkling paper like structure (Fig. 1C).

It is expected that the hydrophobic cavity of β-CD-pyrrole interacts partly with RGO similarly to the well-known host-guest interactions between β-CD-pyrrole and pyrene [18]. After drop-coating of β-CD-pyrrole/RGO solution on a GC electrode, electropolymerization was performed in 0.1 M $LiClO_4$/MeCN by successive potential scanning between 0 and 1.2 V (Fig. 2A). The electrogeneration of poly-[β-CD-pyrrole/RGO] is indicated by the progressive decrease of the irreversible oxidation peak corresponding to the oxidation of the adsorbed pyrrole groups at Ep = 1.1 V. The appearance of a small reversible redox system at $E_{1/2}$ = 0.50 V is attributed to the polypyrrole electroactivity at +0.5 V (Fig. 2A). The weak intensity of this peak system may be ascribed