

# GRAPHENE-BASED HYBRID NANOMATERIALS FOR ELECTROCHEMICAL BIOSENSORS

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**ABSTRACT:** Nanomaterials can be combined or modified with other materials allowing the development of a great variety of composite nanomaterials and nanohybrids with new structural and functional characteristics. This article aims to show the advantages of hybrid nanomaterials as transduction, amplification and labeling elements for the construction of electrochemical biosensing platforms. Special attention will be paid to the use of graphene-based hybrid nanomaterials for biosensing.

**KEYWORDS:** Graphene, graphen oxide, biosensor, dendrimer, hybrid nanomaterial, tyrosinase

**This short paper is dedicated to the 70<sup>th</sup> anniversary of Prof. Dr Sc. Dimitar Stavrev (Technical University- Varna, Bulgaria) for his deep professionalism and knowledge in Material Science and for his over inspiring personality. It is a big honor and privilege to know him.**

## 1. INTRODUCTION

1.1. Electroanalytical chemistry and application of nanomaterials

**Electroanalytical chemistry** is an area of Analytical Chemistry that use the relationship between chemical phenomena involving charge transfer (e.g. redox reactions, ion separation, etc.) and the electrical properties associated to these phenomena, for some analytical determination. It has been empowered with the progress of microelectronics, computer science, software engineering, micro- and nano- lithography, surface chemistry, microfluidics, *materials chemistry* and nanotechnology .

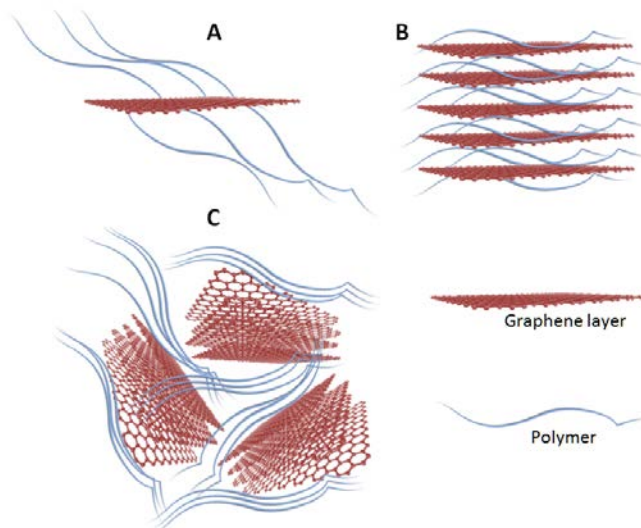
The rational of using nanomaterials in electroanalytical chemistry is well justified by many structural and functional properties of these

### 1.2. Hybrid nanomaterials

It is accepted that nanomaterials are a special type of materials sized between 1 nm and 1  $\mu$ m in at least one dimension. Several authors have intended to define hybrid nanomaterials.[Yamada et al, 1989; Gómez-Romero & Sanchez, 2004; Ashby & Bréchet, 2003; Hagiwara & Suzuki, 2000]. Finally it agreed that *hybrid*

nanosized materials. The most important characteristic is their *high surface area-to-volume ratio*, which determines their unique optical, electroconductive, and magnetic properties. This high surface area favor the large modification of nanostructured electrode surface with chemical receptors and electrocatalysts, but also allows the immobilization of large biomolecule loadings, causing a noticeable increase in the sensitivity of the resulting sensors and biosensors, respectively. As well electroconductive nanomaterials can decrease proteins-nanomaterial distance in biosensors, favoring the direct electron transfer between the redox center of some biomolecules and the bulky electrode material through tunneling mechanisms.

*nanomaterials are not a simple mixture of the starting component materials.* Accordingly, the resulting properties of such nanohybrids are not only the sum of the individual contributions of the component phases, but the role of the inner interfaces could be predominant [Sánchez et al., 1994 New J Chem].



**Fig. 1.** Examples on the structural differences between nanohybrids involving (A) or not involving chemical bonds (B) and nanocomposites (C).

With the aims to provide a **precise description** of these important class of materials, which is based on the previous concepts of Hagiwara and Suzuki [4], we could *define hybrid nanomaterials as the intentional combination of at least a nanomaterial with one or more materials, at an atomic or nanometer-level of mixture, complimenting each other to have new or improved functions and properties which component materials did not possess.*

Nanohybrids can be **classified** according to their *component materials* in: i) inorganic-inorganic, ii) organic-organic, and iii) organic-inorganic hybrid nanomaterials, being the later the most

commonly described in literature. Examples of all these families of nanosized hybrids have been largely employed in electroanalytical chemistry

### 1.3. Hybrid nanomaterials in electroanalytical biosensing technology

The ultimate goal in electrochemical biosensor technology is to design highly specific, robust and cost-effective analytical devices for the accurate and reproducible detection of the great variety of chemical and biochemical compounds with practical interest.

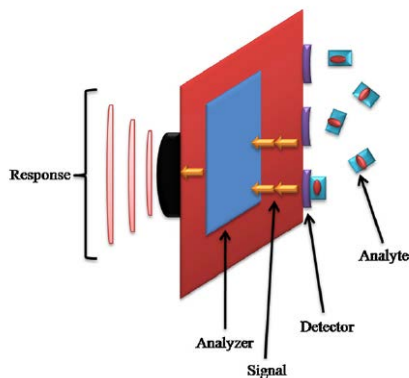


Fig.2. Schematic presentation of a biosensor

To achieve this objective, it is imperative the assembly of sophisticated sensing surfaces having high and specific analyte recognition capacity, and able to ensure the electrocatalytic transformation of the target compound and the fast occurrence of electron transfer processes at the sensing interface.

As previously mentioned, hybrid nanomaterials with inorganic-inorganic, organic-organic and organic-inorganic composition can be tailor-made prepared. Moreover, the number of possible combinations of materials for the synthesis of such nanohybrids with super-functions or new functions is huge. Inorganic building blocks can be composed of nanoparticles, nanotubes, salts and metal complexes, inorganic polymers and layered materials such as clays, xerogels and layered double hydroxides. On the other hand, the number of possible organic building blocks is immeasurable. These novel hybrid nanomaterials can be prepared through different approaches including direct chemical transformation, template and sol-gel synthesis, intercalation, self-assembly and hydrothermal synthesis.

Nanohybrids technology gives us the possibility to design a great variety of novel materials able to provide specific chemical functionalities, accurately arranged at the material surface, for the controlled, stable and oriented immobilization of the analytical

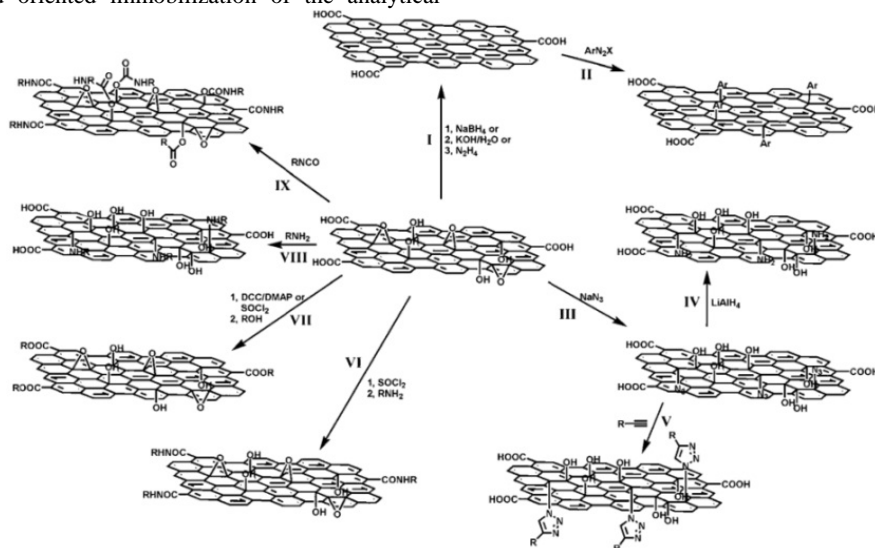


Fig. 3. Examples of covalent functionalization chemistry of graphene or GO. I: Reduction of GO into graphene. II: Covalent surface functionalization via diazonium reaction ( $ArN_2X$ ). III: Functionalization of with sodium azide. IV: Reduction of azide functionalized GO with  $LiAlH_4$ . V: Functionalization of azide functionalized GO through click chemistry ( $R-ChCH/CuSO_4$ ). VI: Modification of GO with long alkyl chains by the acylation reaction. VII: Esterification of GO VIII: Nucleophilic ring-opening reaction of GO. IX: Functionalization of GO with organic isocyanates. (According to Loh et al. 2010).

GO and its derivatives can be easily reduced by chemical, thermal, photothermal, and electrochemical methods, restoring in high yield the structural and electrical conductivity properties of graphene. Graphene and GO have been employed as 2D material component for the preparation of many hybrid and composite materials for sensing and other purposes [Marques et al., 2011]. As a recent example, Yu and co-workers have prepared an amperometric sensor

biomolecules on the sensing interface. In addition, the hydrophilic/hydrophobic patterns of such nanohybrids can be programmed, in advance, to provide analytical biomolecules with a suitable microenvironment for their adequate biochemical function and molecular stability.

## 2. Electrochemical aspects of graphene (G) and graphene oxide (GO).

Since its discovery in 2003, **graphene has emerged** as a new and versatile material for electroanalytical applications [Brownson & Banks, 2010; Pumera et al., 2010; Gan & Hu, 2011]. This wonder material is a million times thinner than paper, stronger than diamond, more conductive than copper. However, graphene is highly hydrophobic, low soluble in water and many organic solvents, and tends to form irreversible agglomerates through strong  $\pi$ - $\pi$  stacking and Van der Waals interactions. Moreover, the absence of chemical functional groups in graphene limits either the easy grafting of chemical receptors, the stable and large immobilization of biomolecules and the modification with organic and inorganic materials through covalent linkages to form advanced nanohybrids.

Chemical derivatization of graphene is helping to overcome these disadvantages. However, the possibility to generically tailor the chemical properties of graphene is limited by its delicate structure, often yielding nanomaterials with poor electroconductive characteristics. For this reason, the intention to prepare soluble and highly functionalized graphene derivatives with low modification of its basal structure, have received considerable attention. Many synthetic approaches commonly use **graphene oxide (GO)**, a water soluble derivative that can be easily prepared by oxidative treatment of graphite, as starting material. GO mainly consists of graphene-like sheets, with hydroxyl and epoxide groups on the basal planes and carbonyl and carboxyl groups at the sheet edges. In addition to the carbon structure of graphene, these oxygen functionalities in GO can be selectively used as **anchoring point** for chemical modification in order to prepare graphene-based hybrid nanomaterials (Fig. 3).

for dopamine by using a nanohybrid of poly-(vinylpyrrolidone)/reduced graphene oxide as transduction surface [Yu et al., 2014].

### 3. Preparation of hybrid reduced GO nanomaterial for biosensing platforms.

Reduced graphene nanoparticles have been prepared from graphene oxide in a two-step covalent modification approach. Graphene oxide was first enriched with reactive epoxy groups by anchoring (3-glycidyloxypropyl)trimethoxysilane at the hydroxyl groups located on the nanocarbon basal plane. Modified graphene oxide was further cross-linked and partially reduced by treatment with the fourth generation ethylenediamine core polyamidoamine G-4 dendrimer producing graphene nanoparticles with crumpled paper-like morphology. This graphene derivative was employed as a coating material for glassy carbon electrodes and the nanostructured electrode was tested for the preparation of electrochemical biosensors by immobilizing the enzyme tyrosinase through cross-linking with glutaraldehyde. This bioelectrode showed excellent electroanalytical behavior for catechol with a fast response in about 6 s, linear range of 10 nM to 22 mM, sensitivity of 424 mA M<sup>-1</sup>, and low detection limit of 6 nM. The enzyme biosensor also showed high stability when stored at 4°C under dry and wet conditions. (E. Araque et al.2013).

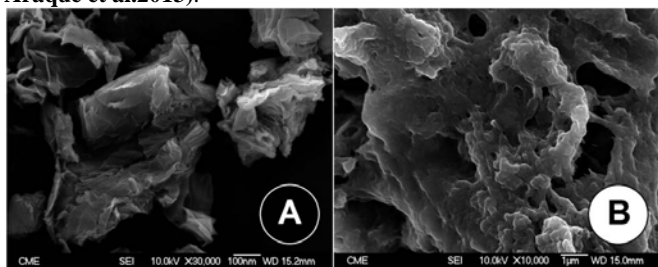


Fig. 4 FE-SEM images of PAMAM-Sil-rGO (A) and Tyr/PAMAM-Sil-rGO (B).

A hybrid nanomaterial was synthesised by covalent attachment of O-carboxymethylcellulose to reduced graphene oxide. Graphene oxide was first anchored with (3-aminopropyl) triethoxysilane moieties to provide reactive primary amino groups at the basal plane. Periodate-oxidised O-carboxymethylcellulose was further covalently attached to this aminated nanomaterial through reductive alkylation with NaBH<sub>4</sub>. Stable aqueous dispersions were obtained with the resulting hybrid nanomaterial, which was used to coat glassy carbon electrodes. Furthermore, the enzyme tyrosinase was covalently immobilised and the nanostructured enzyme electrode was successfully employed for the amperometric detection of catechol in the 20 nM–56 mM range. The biosensor showed excellent analytical performance with a high sensitivity of 270 mA m<sup>-1</sup> and a low detection limit of 0.2 nM. [Elena Araque,[a] Reynaldo Villalonga,\*[a, b] Maria Gamella,[a] Paloma Martinez-Ruiz,[c] Alfredo Sanchez,[a] Valentin Garcia-Baonza,[d] and Jose M. Pingarron\*[a, b] *ChemPlusChem* 2014, 00, 1–9, DOI: 10.1002/cplu.201402017

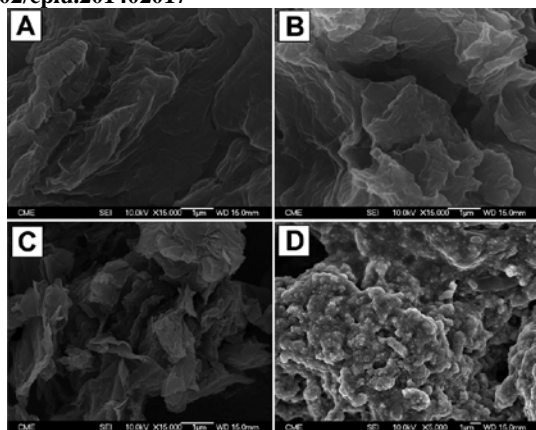


Fig. 5. Representative FE-SEM images of (A) GO, (B) GO-APTES, (C) rGO-CMC and (D) Tyr/rGO-CMC.

A nanostructured architecture for the construction of an amperometric enzyme biosensor toward catechol have been

described. It is based on the electrostatic layer-by-layer assembly of four-generation ethylenediamine core polyamidoamine G-4 dendrimers on glassy carbon electrodes coated with a graphene oxide-carboxymethylcellulose hybrid nanomaterial. This modified surface was further employed for the covalent immobilization of tyrosinase through a glutaraldehyde-mediated cross-linking. The enzyme electrode was used for the amperometric detection of catechol in the 2 – 400 nM range. The biosensor showed excellent analytical performance with high sensitivity of 6.3 A/M and low detection limit of 0.9 nM. The enzyme electrode retained over 93% of the initial activity after 40 days of incubation at 4°C.

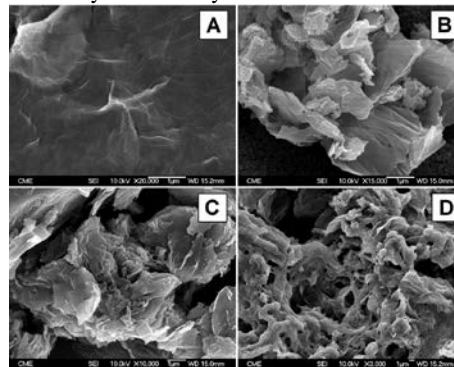


Fig. 6. FE-SEM images of GO (A), GO-CMC (B), PAMAM/GO-CMC (C) and Tyr/PAMAM/GO-CMC (D)

### Conclusions

The application of hybrid nanomaterials in electroanalytical chemistry has **all advantages** cited above for the nanomaterials. However, these relevant functional properties of nanomaterials can be tuned, in an unimaginable fashion, by rational combination with other materials. This fact opens an exciting door for sensor and biosensor technology, which is well supported by the advances in other areas such as surface science, organic and inorganic synthesis, polymer and biomolecular chemistry and nanotechnology. Nanohybrids technology gives us the possibility to design a great variety of novel materials able to provide specific chemical functionalities, accurately arranged at the material surface, for the controlled, stable and oriented immobilization of the analytical biomolecules on the sensing interface. In addition, the hydrophilic/hydrophobic patterns of such nanohybrids can be programmed, in advance, to provide analytical biomolecules with a suitable microenvironment for their adequate biochemical function and molecular stability.

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