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Graphene for electrochemical sensing and biosensing

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Graphene has proved to be an excellent nanomaterial for applications in electrochemistry. We review progress in constructing high-performance electrochemical sensors and biosensors. We also discuss:

- **different routes for graphene fabrication;**
- **graphene-modified electrodes and graphene-composite electrodes for sensing, including those based on ionic liquids;**
- **incorporation of biorecognition elements into graphene-based electrodes; and,**
- **graphene-supported electrocatalytic nanoparticle-based electrochemical sensors and biosensors.**

Keywords: Biorecognition; Biosensor; Carbon nanotube; Detection; Electrochemical sensing; Electrochemistry; Graphene; Nanomaterial; Nanoparticle; Sensor

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1. Introduction

Graphene-based nanomaterials has captured great interest among physicists, chemists and materials scientists alike. Graphene is a two-dimensional (2-D) sheet of carbon atoms in a hexagonal configuration with atoms bonded by sp^2 bonds. These bonds and this electron configuration are the reasons for the extraordinary properties of graphene, which include a very large surface area [at 2630 m^2/g, it is double that of single-walled carbon nanotubes (SWCNTs)], a tunable band gap, room-temperature Hall effect, high mechanical strength (200 times greater than steel), and high elasticity and thermal conductivity [1].

Graphene is the most recent member of the multi-dimensional carbon-nanomaterial family, starting with fullerenes as a 0-D material, SWCNTs as 1-D nanomaterials, and ending with graphite as a 3-D material. Graphene fills the gap for 2-D carbon nanomaterials (Fig. 1). Isolation of individual graphene sheets was long sought, but only in 2004 it was achieved by a surprisingly simple technique [2]. Since then, fundamental research and research on applications have increased rapidly. While single-layer graphene is extremely interesting, similarly interesting, unique properties are also offered by bi-layer and multi-layer graphene [called stacked graphene platelets (GNPs)] [3].

Graphene is an ideal material for electrochemistry [4–7] because of its very large 2-D electrical conductivity, large surface area and low cost. In comparison with CNTs, two advantages of graphene are apparent, as follows.

(1) Graphene does not contain metallic impurities as CNTs do [8]. In many cases, such impurities dominate the electrochemistry of CNTs (so far, such negative influence is known for hydrazine [9,10], hydrogen peroxide [11,12], halothane [13], glucose [14], amino acids [15], and short regulatory peptides [16] even at <100 ppm levels of impurities in CNTs [17]) and lead to misleading conclusions.
(2) The production of graphene uses graphite, which is cheap and accessible.

Here, we review the use of graphene in electrochemical sensors and biosensors. This area is particularly interesting, with the first articles emerging in 2008. Since then, their number has grown explosively.

2. Synthesis

To understand the electrochemical properties of graphene, one must first

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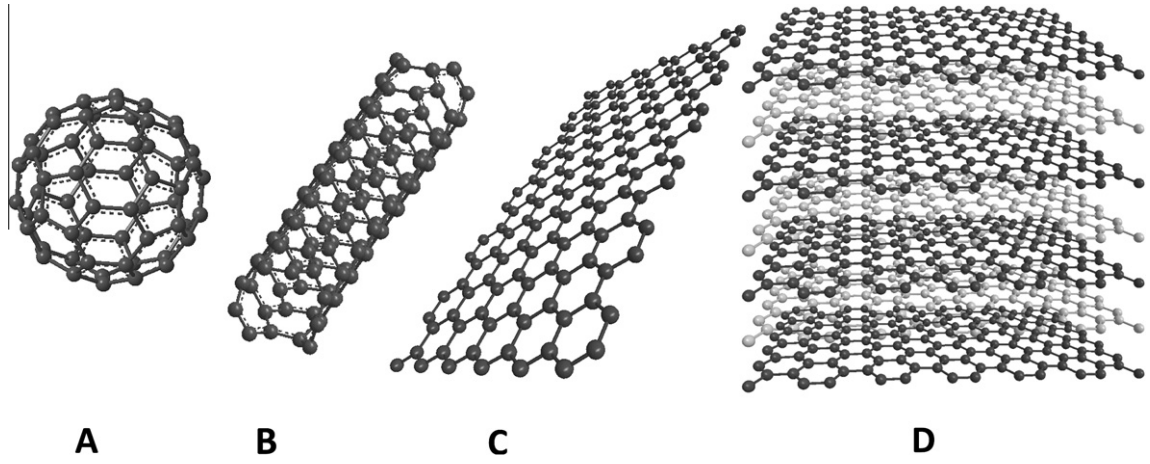

Figure 1. Forms of sp^2-bonded carbon. (A) Fullerene, (B) Single-walled carbon nanotubes, (C) Graphene, (D) Graphite.

understand the way graphene is synthesized. Graphene can be prepared in several ways but only some are suitable for electrochemical applications for sensing and biosensing.

A) Graphene can be prepared by ''peeling-off'' highly oriented pyrolytic graphite (HOPG) [2].
B) Graphene can be epitaxially grown on silicon wafers.

These two methods are useful for studying the electronic properties of graphene. However, to study the electrochemistry of graphene and apply graphene in electrochemical-sensing systems, bulk quantities of graphene are required [18] and can be produced as follows.

C) Chemical vapor deposition (CVD) [19]. This method often results in multi-layered structures.
D) Intercalation of small molecules in a graphite lattice and its exfoliation by thermal shock [20] or using ultrasonication [21].
E) ''Unzipping'' CNTs via different routes. It is possible to use hypermanganate chemical oxidation of CNTs, which open up after the treatment [22], or plasma etching [23] of multi-walled CNTs (MWCNTs) to produce graphene nanoribbons. Here, it should be mentioned that graphene synthesis from MWCNTs brings with it the danger of introducing metallic impurities intercalated in MWCNTs with all sorts of negative consequences leading to the unpredictable electrochemical behavior of such materials as well as toxicity. Therefore, these methods should be avoided when using graphene for electrochemistry unless the MWCNTs are free of impurities.

We need to mention that these methods for ''bulk'' scale production of graphene usually produce most (~99%) of the material as multi-layer GNPs and only about 1% as true monolayer graphene sheets [21]. Only recently, novel methods were introduced, resulting in yield of graphene monolayers of about 90%. We also need to emphasize that individual graphene layers tend to stack to form multi-layered nanostructures [21].

From the discussion on the methods of synthesis, it is clear that, if bulk quantities of graphene prepared by any of the above methods are used, the majority of the material has a multi-layered structure and should be regarded as GNPs instead of graphene. The electrochemistry of GNPs is as interesting and as important as that of graphene. However, before describing the material as ''graphene,'' very careful material characterization should be carried out. In the following text, we use the terms graphene or GNPs as used by the authors of the particular paper referred to. However, it should be noted that, in all cases, the materials are graphenes with a multi-layer structure.

3. Electrochemical properties

The structure of highly oriented pyrolytic graphite (HOPG) is multi-layer graphene with very few defects. Although the edge plane of a graphene sheet has an electron transfer-rate constant (k_e) of ~0.01 cm/s, the basal plane is, in effect, inert electrochemically (k_b is below 10^{-9} cm/s). The basal plane of a graphene sheet can contain defects but the defects are generally considered to be edge-plane sites because of their fast electron-transfer kinetics. The defect-free graphene sheets had a k_b that was near zero [24].

As the electrochemistry of graphene sheets is driven by its edges (either in planar graphene or in rolled-up graphene – CNTs) where heterogeneous electron transfer (HET) is fast, when looking at the structure of CNTs and graphene (see Fig. 1), one might expect higher observed HET on graphene sheets simply because there is a larger number of edges per mass of the material. However, only

three articles focus on this comparison, both concluding what is obvious from structural observation. We proved that the observed HET rate of carbon nanotube materials is comparable to that of graphite only because CNT materials contain large quantities of nanographite impurities. When pure carbon nanotubes are used, the observed HET rate is actually significantly lower than that of graphitic materials. In other words, most of the electrochemical activity of carbon nanotubes is due to the presence of the *nanographite impurities* contained within them [25]. Alwarappan et al. [26] compared the electrochemistry of SWCNTs and graphene using cyclic voltammetry and differential pulse voltammetry (DPV). They tested several electroactive compounds [e.g., ferro/ferricyanide, ascorbic acid (AA), serotonin (ST) and dopamine (DA)] and found out that, due to fast HET on graphene, the DPV voltammogram of a mixture of AA, ST and DA provided well-resolved oxidative peaks, while, due to slower HET, SWCNTs provided one broad signal. This shows a very significant advantage in electrochemical sensing of neurotransmitters for graphene-based electrodes over SWCNTs. We found similar conclusions for case of stacked graphene nanofibers (SGNFs) versus MWCNTs for various biomarkers [27]. In addition, we also found that the electrochemical response for selected biomarkers in the case of SGNFs is about twice that of MWCNTs as electrode material [27]. Very recently, we compared results in the performance of an analytical electrochemical method for determination of free DNA bases (guanine, adenine, thymine and cytosine) and DNA strand-related influenza A (H1N1) [28]. We demonstrated that the sensitivity of the graphene-based electrode was about 2–4 times greater than that of MWCNTs. These reports clearly demonstrate the superior performance of graphene-based electrodes compared to that of (impurity-free) CNTs. We demonstrated that the electrochemical response of graphene sheets is independent of the number of layers from a single graphene sheet to multi-layer stacked graphene nanoribbons for dopamine and ascorbic acid [29]. We also demonstrated that multi-layer graphene nanoribbons exhibit larger capacitance than their few-layer and single-layer graphene counterparts [30].

4. Applications as electrochemical sensors and biosensors

The following sub-sections highlight important applications of graphene in sensing and biosensing. The first sub-section (4.1.) focuses on graphene as a transducer for electrochemical sensing in the form of electrodes modified with graphene powder or graphene-composite electrodes. Sub-section 4.2. focuses on electrochemical biosensing. The different methods of incorporating bio-recognition elements in graphene-based electrochemical sensing devices are discussed. In sub-section 4.3., we illustrate that graphene can be used efficiently as a conducting surface with a very high surface area for the deposition of catalytic nanoparticles (NPs) and consequent electrochemical sensing. We summarize quantitative parameters of the sensors and biosensors (when available) in Table 1.

It is possible to see from data presented in the Table 1 that similar forms of graphene provide similar analytical performance (e.g., analysis of dopamine performed on graphene sheets exhibits the almost the same linear range (5–200 μM [34] versus 4–100 μM [35])). The small differences presumably arise from slight differences in the preparation method. Although both groups used modified Hummer's method, the lack of experimental details on graphene preparation prevents insight into the reasons for the differences. We stress the importance of providing complete experimental details of graphene preparation in future works in order to have comparable data. Similarly, detection of glucose on reduced graphene oxide (GO) [31] and GO [45] exhibited similar analytical parameters {e.g., limits of detection (LODs) were

Table 1. Summary of relevant quantitative parameters of selected sensors and biosensors

Analyte	Electrode material[a]	Limit of detection	Linear range	Ref.
Pb^{2+}	Graphene	0.02 μg/L	0.5–50 μg/L	[33]
Cd^{2+}	Graphene	0.02 μg/L	1.5–30 μg/L	[33]
H_2O_2	Graphene/AuNPs/chitosan	180 μM	0.2–4.2 mM	[53]
H_2O_2	Reduced graphene oxide	0.05 μM	0.01–10 mM	[31]
Dopamine	Graphene	NA	5–200 μM	[34]
Dopamine	Graphene	2.64 μM	4–100 μM	[35]
NADH	Ionic liquid-graphene	5 μM (ethanol)	0.25–2 mM	[36]
Glucose	Graphene/Au/Nafion	5 μM	0.015–5.8 mM	[51]
Glucose	Reduced graphene oxide	2 μM	0.01–10 mM	[31]
Glucose	Graphite nanosheet/Nafion	NA	0.2–1.4 mM	[41]
Glucose	N-doped graphene	0.01 mM	0.1–1.1 mM	[44]
Glucose	Graphene oxide	1 μM	1–20 μM	[45]

[a]– As noted in Section 2, all materials used and named as ''graphene'' are multi-layered graphene platelets. However, we use terms that authors used.

2 μM and 1 μM, respectively}. In other cases, direct comparison was not possible as the graphene-based materials were in composite mixtures with polymer binder and/or catalytic NPs.

In the following three sub-sections, we discuss particular articles in greater detail. We always add information (if available) on how the material characterization of graphene-based materials was carried out, as this is crucial to judge the validity and the importance of a particular report.

The sub-sections are: (4.1.) electrochemical sensors; (4.2.) electrochemical biosensors; and, (4.3.) graphene-supported NPs for electrochemical detection. Even though in the recent year there is the undesirable trend in electroanalytical articles (e.g., [19]) of calling any electrochemical sensor detecting a biologically important compound (e.g., neurotransmitter, cofactor of enzyme, DNA base or free DNA strand) an ''electrochemical biosensor'', we follow the IUPAC recommendation and call such a sensor just ''sensor'', as IUPAC defines ''electrochemical biosensor'' as follows: ''Electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element'' [31]. Therefore, the only sensors that we call electrochemical biosensors integrate a biorecognition element into the sensor.

4.1. Electrochemical sensors

Papakonstantinou and co-workers [19] were the first researchers to use graphene-based nanomaterials for electrochemical sensing. They grew GNP films on silica substrates using a catalyst-free method. GNPs have a thickness of several tens of nanometers and thus contain several hundred stacked graphene sheets. The authors should be credited for very careful characterization of the prepared material by means of high-resolution transmission electron microscopy (TEM), scanning electron microscopy, energy dispersive X-ray spectroscopy, and X-ray photoelectron spectroscopy. GNPs demonstrated fast electron-transfer kinetics for the ferro/ferricyanide couple and very good performance for simultaneous

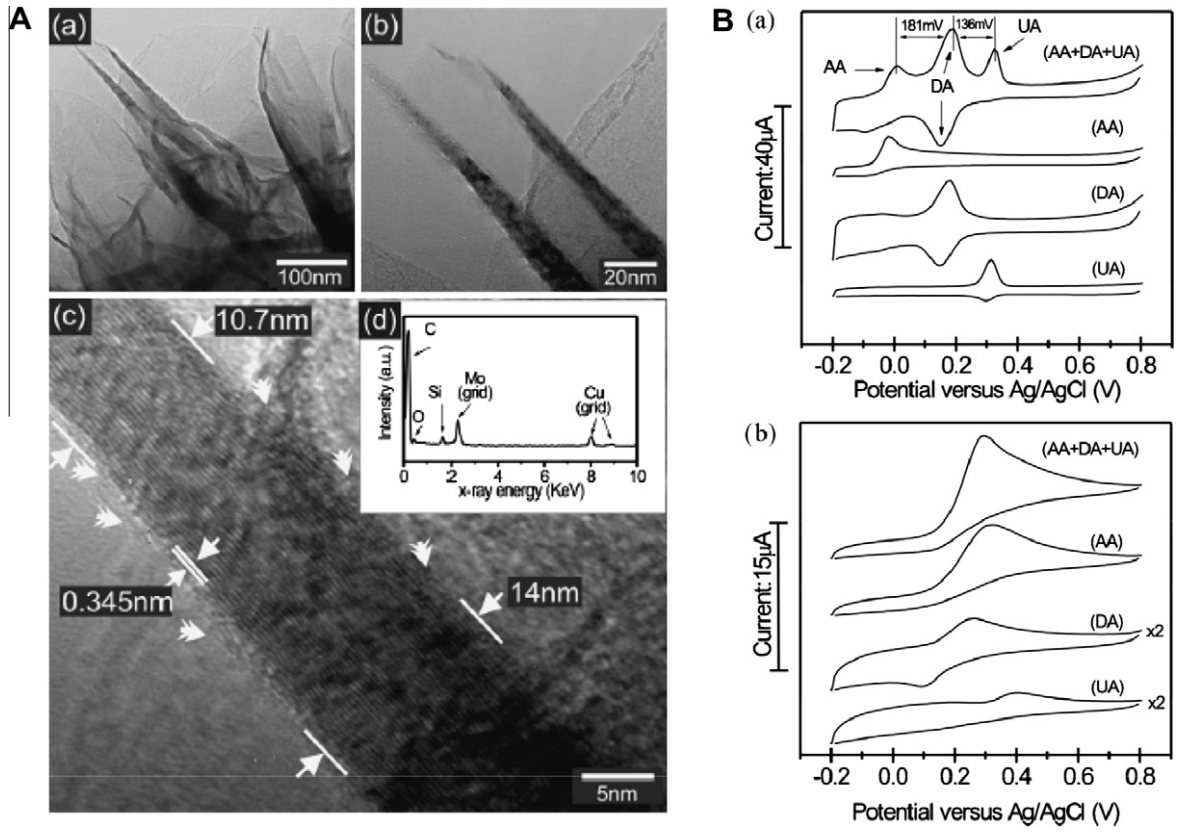

Figure 2. Graphene platelets (GNPs) for electrochemical sensing. (A) (a) and (b) Transmission electron microscopy (TEM) images of GNPs at different magnifications; (c) high-resolution TEM image of GNPs showing a nanoflake with a knife-edge or conical structure with open graphitic planes; (d) energy-dispersive X-ray spectrum, showing the chemical composition of GNP films. (B) (a) and (b) Cyclic voltammetry profiles of GNPs and bare glassy-carbon electrodes, respectively, in a solution of 50 mM, pH 7.0 PBS buffer containing 1 mM ascorbic acid, 0.1 mM dopamine, and 0.1 mM uric acid (Reprinted with permission from [19]).

determination of dopamine, ascorbic acid, and uric acid. The electrochemical performance of GNPs was superior to that of a glassy-carbon electrode (GCE) but comparable to an edge-plane pyrolytic graphite (EPPG) electrode (Fig. 2).

Similarly, Dong and co-workers [32] studied the electrochemical properties of reduced GO in great detail. GO platelets were about 1 nm thick, as characterized by atomic force microscopy (AFM), thus containing 2–3 graphene layers. The electrochemical probes used included potassium ferricyanide, guanine, adenine, thymine, cytosine, H_2O_2/β-nicotinamide adenine dinucleotide, dopamine, ascorbic acid, uric acid, and acetaminophen. The performance of a GO-modified GCE was compared to that of a graphite-modified electrode and a bare GCE. There were different analytical characteristics for detection of dopamine in the work of Papakonstantinou et al. [19] and Dong et al. [32]. This is because, as mentioned, the GNPs used in [19] contained several tens of stacked graphene sheets while the GNPs in [32] had only a few layers.

Similar to the work of Papakonstantinou [19], Li and co-workers [33] used graphene-based nanomaterials for the sensitive detection of dopamine in the presence of ascorbic acid. The authors demonstrated well-resolved peaks of dopamine and ascorbic acid on graphene while, on GCEs, these peaks overlapped. However, the ''graphene'' in this work was prepared by a method similar to the one above. As expected, the authors' own TEM images revealed a multi-layered structure for the ''graphene.'' Thus, the ''graphene'' comprised GNPs and the electrochemical response was again different in the different articles. In future, careful work comparing graphene with different numbers of layers is needed to provide insight into how the number of graphene layers in multi-layer structures influences the electrochemistry.

Similarly, Kim et al. [34] discussed the use of graphene for electrochemical detection of dopamine in the presence of ascorbic acid and compared the results from a GCE and a graphene-modified electrode. They concluded that graphene showed a faster HET rate than a GCE by itself. In this case, the graphene nanomaterial was prepared by Hummer's method but no detailed characterization was provided.

Wang et al. used a graphene-modified electrode for the electrochemical detection of cadmium [35] and cadmium and lead [36]. The authors claimed a higher level of sensitivity toward the detection of heavy metals when compared to a bare GCE. The graphene was prepared by the ultrasonication of graphite oxide and reduction of the resulting material using hydrazine. As mentioned above, such a method results in materials that contain 99% multi-layered structures and 1% graphene. According to the AFM measurements published in Wang's work, the thickness of the flakes was about 3–4 nm, which corresponds to about 10 graphene layers, so the material should have been called GNP.

Niu and co-workers [37] prepared an ionic liquid (IL)-graphene/chitosan-modified GCE. Such a composite can provide stable, low-potential amperometric detection of the reduced form of nicotinamide adenine dinucleotide (NADH). The IL-graphene/chitosan film offers a remarkable decrease in the overvoltage of the NADH oxidation and eliminates surface-fouling effects. A very simple ethanol biosensor has been constructed successfully, demonstrating the potential application of IL-graphene nanocomposites. In this case, the graphene was prepared by Hummer's method but no characterization was provided.

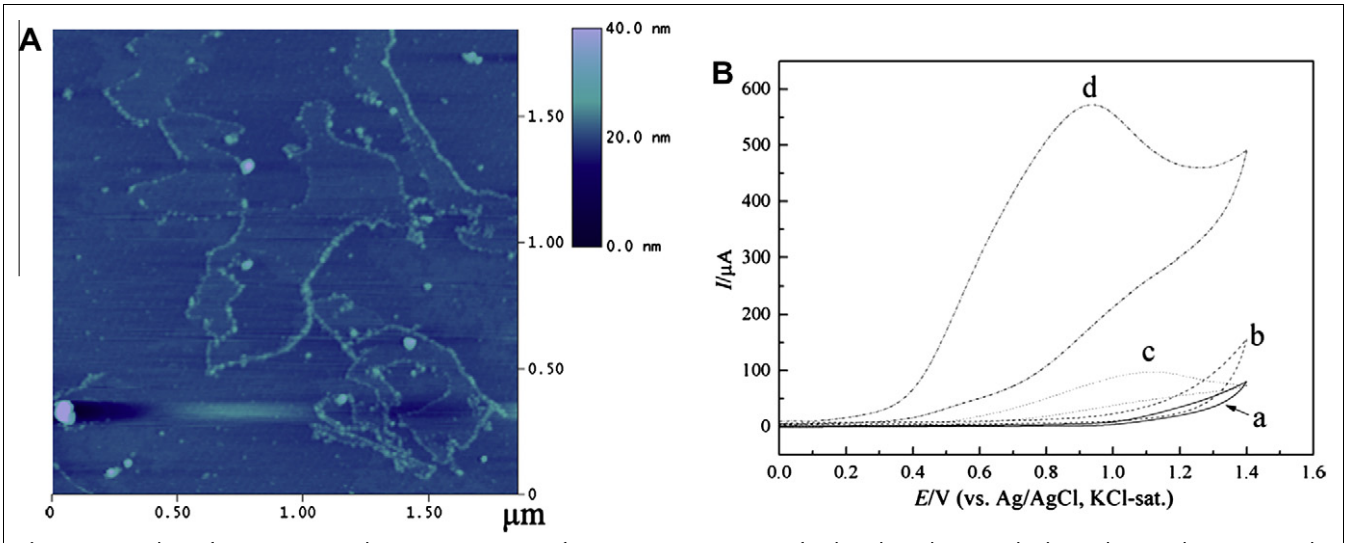

Figure 3. Graphene for environmental sensing. (A) Atomic force microscopy image of reduced graphene oxide sheets deposited on a mica substrate. (B) Cyclic voltammograms at (a and c) the bare glassy-carbon electrode (GCE), and (b and d) reduced graphene sheets/GCE in the absence (a and b) and presence (c and d) of 10 mM hydrazine in 0.1 M KOH solutions at a scan rate of 0.1 V/s (Reprinted with permission from [37]).

Zhang et al. [38] produced reduced graphene sheets for the electrocatalytic oxidation of hydrazine in alkaline media. They demonstrated that reduced graphene (which should be called reduced GO [39]) exhibits lower over-potentials for hydrazine oxidation than GCEs. In this case, the ''graphene'' was prepared by Hummer's method and characterized by AFM and Raman spectroscopy (see Fig. 3).

Lin et al. [40] fabricated an electrochemical sensor based on the electrocatalytic activity of functionalized graphene for the sensitive detection of paracetamol. The electrochemical behavior of paracetamol on graphene-modified GCEs was investigated by cyclic voltammetry and square-wave voltammetry. This work demonstrated that a graphene-modified electrode exhibits electrocatalytic activity to paracetamol. A quasi-reversible redox process of paracetamol at the modified electrode was obtained, and the over-potential of paracetamol decreased significantly compared with that at the bare GCE. In this work, the graphene was prepared by a modified Brodie method and characterized by TEM. From the TEM observations, the graphene structure was a single layer to a few layers.

Li et al. [26] demonstrated that the density of negative charge present on the graphene surface was greater than that found in SWCNTs. In addition, the possibility of

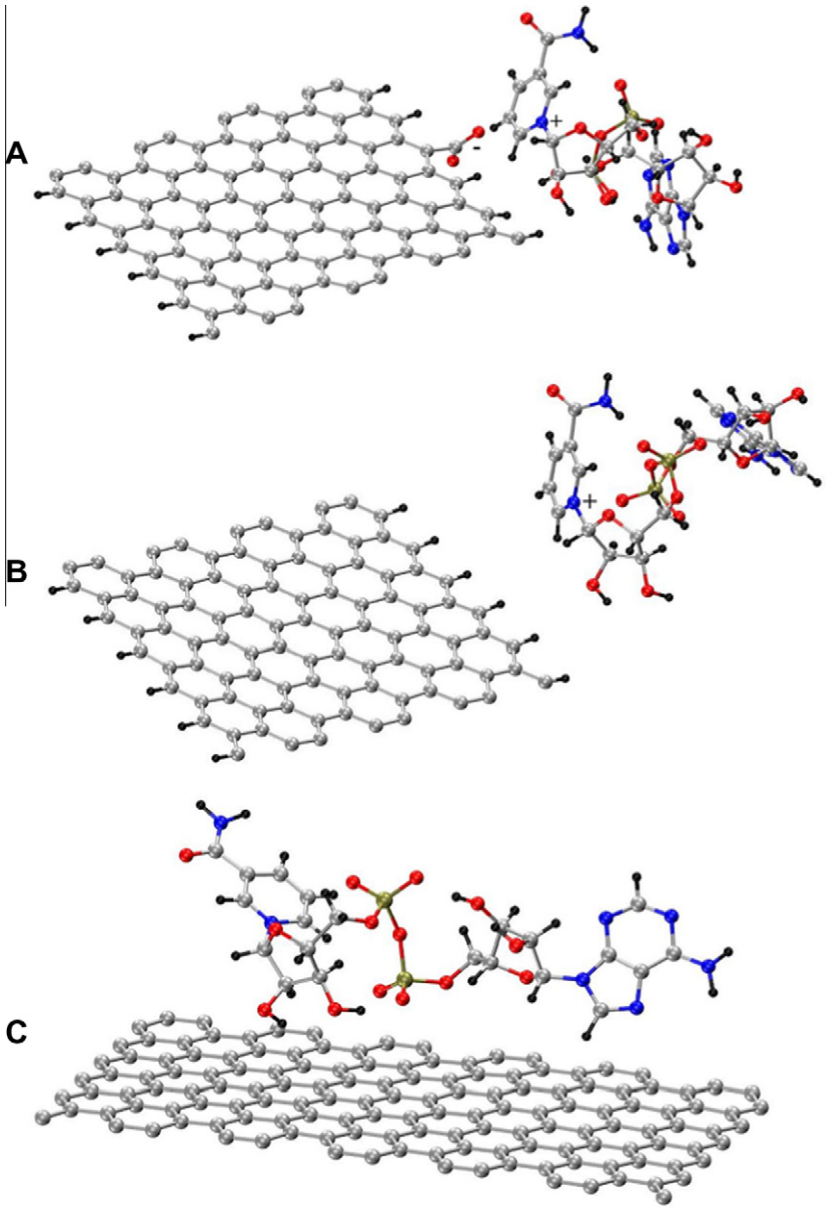

Figure 4. Adsorption of nicotine adenine on graphene sheets. Geometries for adsorption of NAD^+ on (A) graphene edge terminated by hydrogen atoms and containing one $–COO^-$ group, (B) graphene edge fully terminated by hydrogen atoms, and (C) basal plane of a graphene sheet via Car-Parrinello molecular dynamics (CPMD). Gray, C; blue, N; red, O; yellow, P; black, H (Reprinted with permission from [40]).

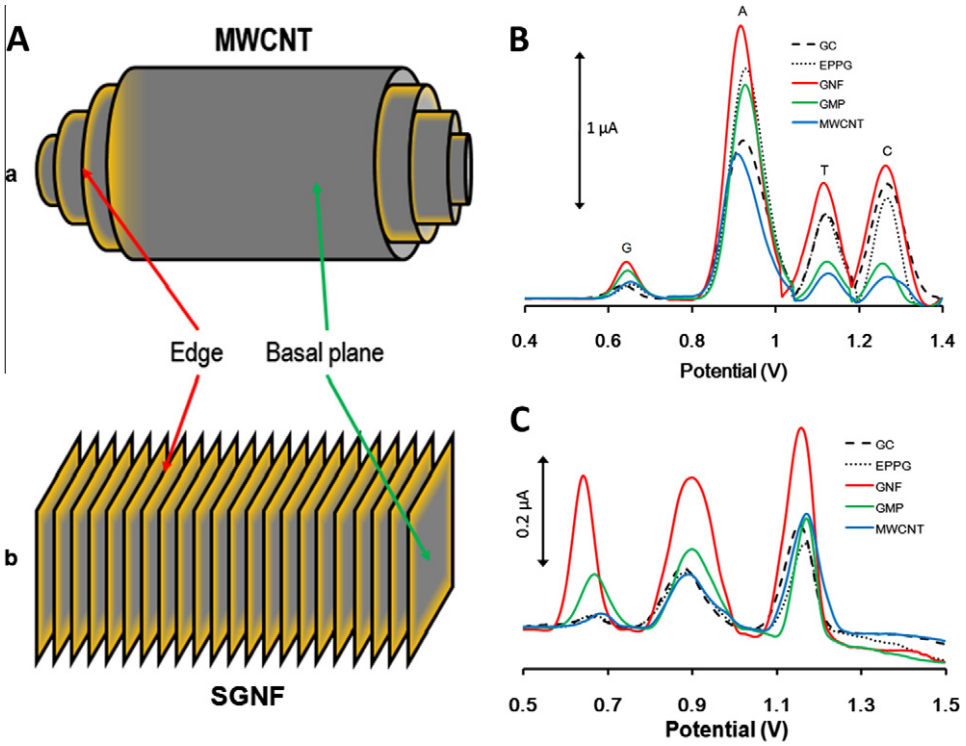

Figure 5. Stacked graphene nanofibers (SGNFs) outperform carbon nanotubes and graphite in the detection of free DNA bases and A (H1N1) DNA strand. (A) Graphene-sheet orientation in multi-walled carbon nanotubes (MWCNTs) (a) and SGNFs (b). The highly electroactive edge portion of the sheets is represented in yellow. (B) differential pulse voltammograms (DPVs) for a mixture of guanine, adenine, thymine, and cytosine at SGNF (red), graphene platelet (GNP) (green), and MWCNT (blue) electrodes. For comparison, glass-carbon electrode (GCE) (black dashed) and edge-plane pyrolytic graphite (EPPG) (black dotted) electrode signals are also shown. (C) DPVs for ssDNA A (H1N1) at SGNF (red), GNP (green), and MWCNT (blue) electrodes. For comparison, GCE (black dashed) and EPPG (black dotted) electrode signals are also shown (Reprinted with permission from [28]).

employing graphene for the electrochemical detection of important neurotransmitters (e.g., dopamine and serotonin) was evaluated and compared with that of SWCNTs. In all these experiments, graphene exhibited a better sensitivity, signal-to-noise ratio, and stability than SWCNTs. In addition, the graphene electrodes exhibited superior biosensing performance than SWCNTs toward dopamine detection in the presence of common interfering agents (e.g., ascorbic acid and serotonin).

Experimentally and theoretically, we demonstrated the reasons for, and the mechanism of, the adsorption of β-Nicotinamide Adenine Dinucleotide (NAD^+) on graphene sheets [41]. NAD^+ is a foremost element in electrochemical enzyme biosensors and biofuel cells that employ dehydrogenase enzymes. NAD^+ adsorption on most carbon materials (e.g., CNTs and graphite) has not been understood well and presents critical problems. We demonstrated that NAD^+ adsorption at sp^2-carbon materials is due to oxygen-containing groups, specifically carboxylic groups, which form on graphene sheets at the edges and edge-like defects. These oxygen-containing groups are generally present in graphene sheets because of spontaneous oxidation in air. Using amperometry, X-ray photoelectron spectroscopy (XPS), and cyclic voltammetry, we were able to show that NAD^+ adsorption and electrode passivation occur at the edges and edge-like defects of graphene. Using XPS and Car-Parrinello molecular dynamics (CPMD), we proved that, if a positive NAD^+ is close to a graphene sheet edge containing a $–COO^-$ group, there is a significant interaction that agrees with experimental results. By contrast, no significant interactions were observed when NAD^+ was located near the basal plane of the graphene or near hydrogen-only substituted edges of graphene sheets (Fig. 4).

The use of reduced GO in the electrochemistry of biomarkers was studied comprehensively. Free-DNA bases (adenine, cytosine, guanine, and thymine), oxidase/dehydrogenase-related molecules (hydrogen peroxide/NADH), neurotransmitters, uric acid, ascorbic acid, and acetaminophen were employed to study electrochemical responses on GO that had been chemically reduced [31]. Reduced GO displays a considerably larger electrochemical response toward the oxidation of these probes than that of graphite or GCEs. As mentioned before, such enhanced response is associated with the presence of groups containing oxygen on the surface of the reduced GO.

Because the results of the structure of the reduced GO nanomaterials are uncertain and the electronic characterization of the GNPs (including HET rate) no longer depends on thickness, study of more well-defined materials would be preferable in terms of electrochemistry and electrochemical sensor and biosensor fabrication. SGNFs can now be purchased in bulk, whereas graphene and GNPs cannot. The nanofibers of the graphene sheets have a perpendicular orientation relative to the long axis of the fiber. This causes the presence of a large number of edge-plane sites. This orientation means the material is chemically and electrochemically unique, as only the edges are exposed, in contrast to usual graphite crystals or CNTs. In fact, SGNFs are structural opposites of CNTs because almost only the edges of the graphene sheets are exposed (see Fig. 1). Since the electrochemistry of sp^2 materials is established at edge-plane sites, the electrochemical properties of SGNFs should be extraordinary compared with graphite microparticles and CNTs. SGNFs have rapid electron-transfer rates for a wide variety of compounds including ascorbic acid, dopamine, $FeCl_3$, ferrocyanide, uric acid, as well as the reduced form of β-NAD. SGNFs have properties that are electrochemically superior to those of graphite microparticles and MWCNTs because of their high percentage of graphene-sheet edges [27].

We showed that SGNFs demonstrated superior electrochemical performance for oxidation of DNA bases compared with CNTs [28]. This is due to an exceptionally large number of accessible graphene-sheet edges on the surface of the nanofibers when compared to CNTs, as shown by TEM and Raman spectroscopy. The oxidation signals of adenine, guanine, cytosine, and thymine exhibit currents 2–4 times greater than on CNT-based electrodes. SGNFs also exhibit higher sensitivity than edge-plane pyrolytic graphite electrodes, GCEs, or graphite microparticle-based electrodes. We also demonstrate that influenza A (H1N1)-related strands can be sensitively oxidized on SGNF-based electrodes, which could therefore be applied to label-free DNA analysis (see Fig. 5).

4.2. Electrochemical biosensors

Chen and co-workers used graphite platelets (with a thickness under 100 nm) for preparing a glucose biosensor based on a mixture of GNPs, Nafion binder, and glucose oxidase (GOx) [42]. The authors claim to have observed direct electron transfer from glucose to the GNPs.

GO has been successfully employed in bioelectrochemistry [43]. GO supports efficient electrical wiring of the redox centers of several metalloproteins containing heme (cytochrome *c*, myoglobin, and horseradish peroxidase) to the electrode (see Fig. 6). It is meaningful to note that proteins retain their structural integrity and biological activity when they form mixtures with GO [42]. These features predict promising applications for GO/protein complexes in biosensor and biofuel-cell development.

Niu et al. [44] prepared graphene sheets protected by a polyethylenimine-functionalized IL, which could be

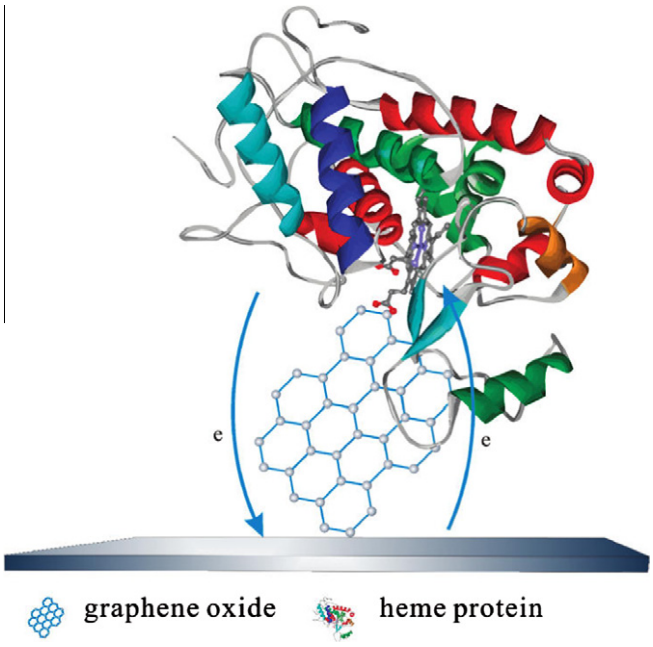

Figure 6. Wiring of proteins by graphene for electrochemistry. Graphene-oxide (GO)-supported heme proteins at the surface of glassy-carbon electrodes (for convenience, oxygenated functional groups at the surface of GO are not shown) (Reprinted with permission from [42]).

stably dispersed in water, and exhibited high electrocatalytic activity toward the reduction of O_2 and H_2O_2. Because of their favorable electronic properties and biocompatibility, graphene-based composites accomplished

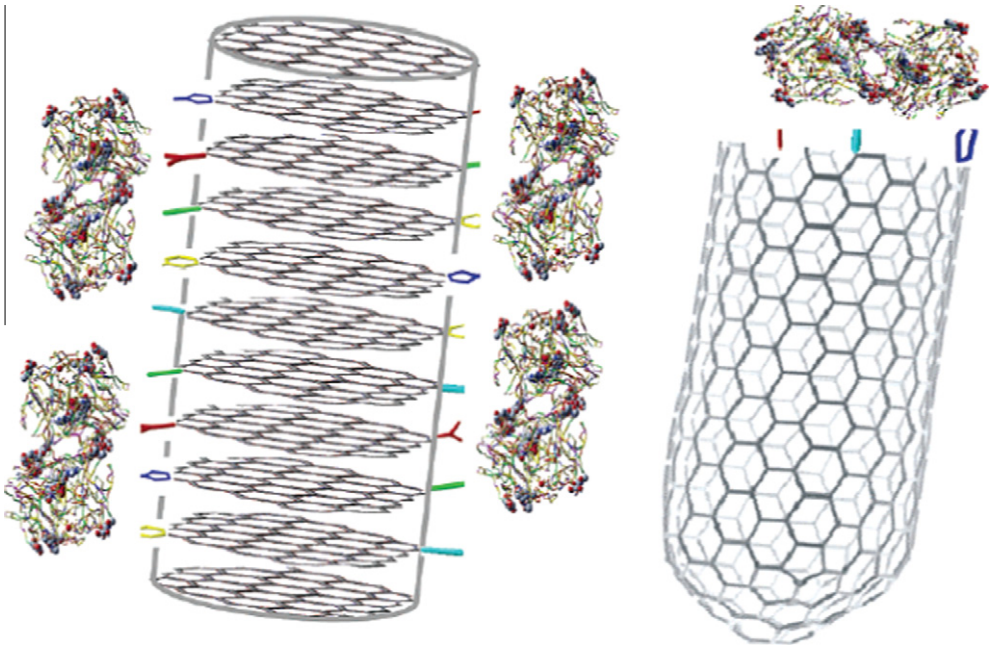

Figure 7. Immobilization of the model enzyme glucose oxidase on stacked graphene platelet nanofibers and single-walled carbon nanotubes (Reprinted with permission from [47]).

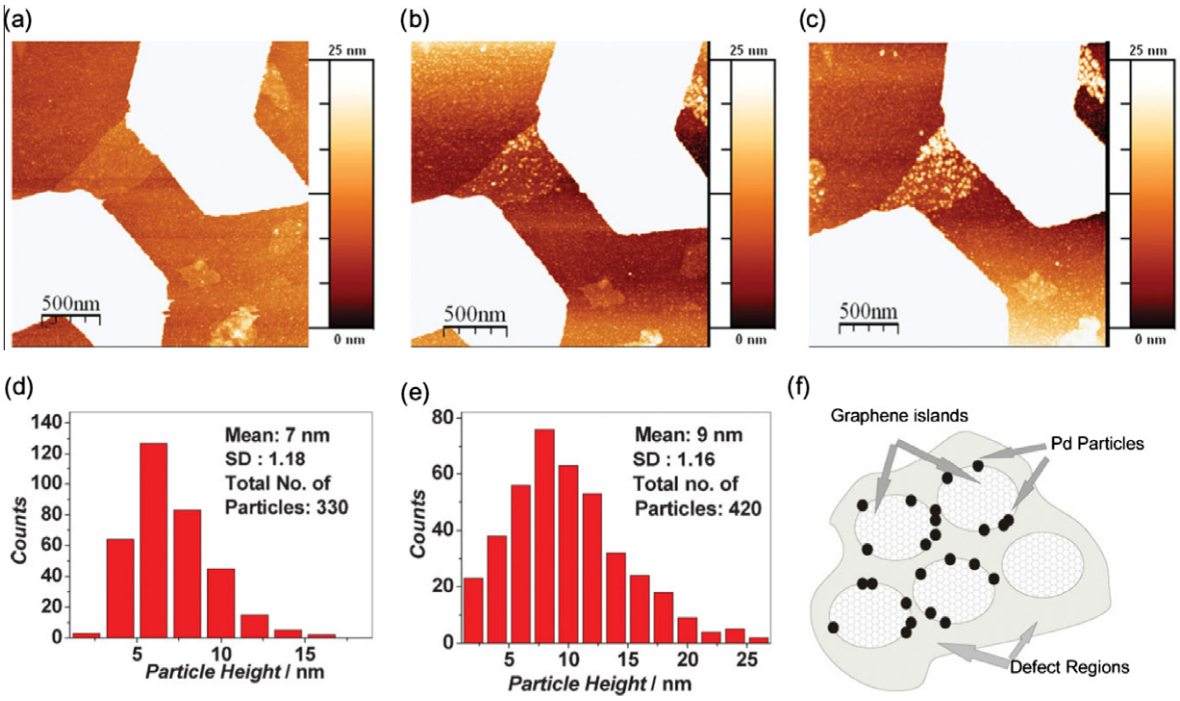

Figure 8. Nanoparticle-decorated graphene sheets for electrochemistry. Atomic force microscopy images of an individual graphene sheet contacted by two electrodes at various stages: a) initial bare, unmodified layer; b, c) after electrodeposition of Pd at –0.70 V and –0.85 V versus Pt, respectively. d, e) Histograms showing the size distribution of particles in (b) and (c), respectively. f) Possible nucleation sites for Pd at defects at the edges of intact graphene islands (Reprinted with permission from [49]).

the direct electron transfer of redox enzyme while maintaining a good level of bioactivity. They fabricated an electrochemical glucose biosensor based on such polymer-protected graphene/polyethylenimine-functionalized IL nanocomposites, which showed potential for further fabrication of real-life biosensors. The authors claimed direct electron transfer from the GOx via the graphene sheets.

Lin et al. [45] doped graphene sheets with nitrogen atoms via nitrogen plasma treatment of graphene synthesized via a chemical method. In combination with GOx enzyme, it was shown that an N-doped graphene-based biosensor can detect glucose in the presence of interferences down to 10 μM concentration. This is comparable to LODs of glucose using chemically-reduced GO [31].

Qu et al. [46] showed that GO possesses intrinsic peroxidase catalytic activity due to the presence of carboxylic groups at the edges of the graphene sheets, so there is no need for GOx enzyme, and such a biosensor

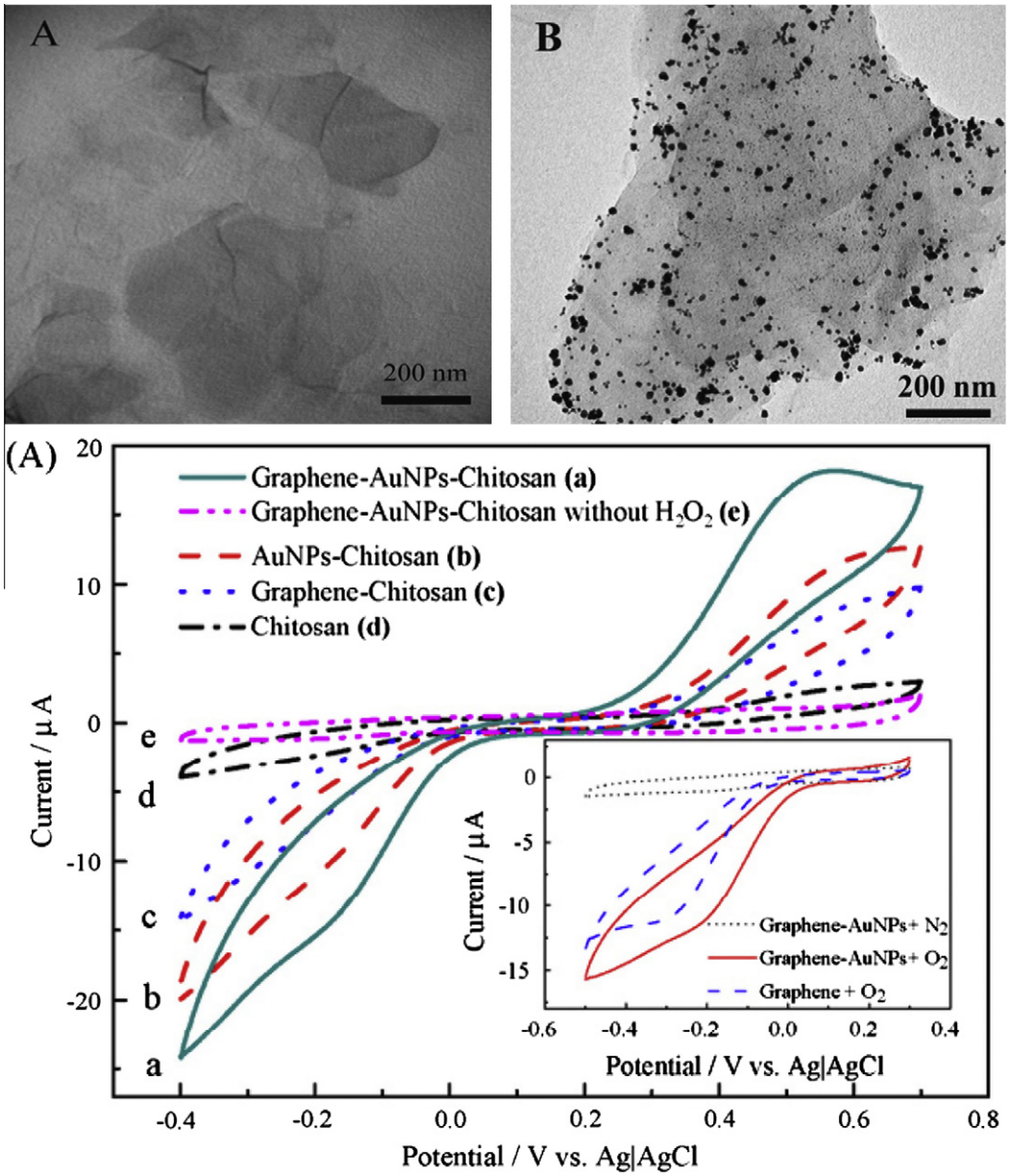

Figure 9. Nanoparticle-decorated graphene for enzymatic biosensing. Transmission electron microscopy images of (A) polyvinylpyrrolidone-protected graphene and (B) gold-nanoparticle (AuNP)-decorated graphene. (C) Cyclic voltammograms of (a) graphene/AuNPs/chitosan, (b) AuNPs/chitosan, (c) graphene/chitosan, and (d) chitosan-modified electrodes in N_2-saturated phosphate buffer (0.05 M, pH 7.4) containing 2.5 mM H_2O_2, and graphene/AuNPs/chitosan-modified electrode (e) in N_2-saturated phosphate buffer at a scan rate of 0.05 V/s. Inset: Cyclic voltammograms of graphene/chitosan (dashed, blue), graphene/AuNPs/chitosan-modified electrodes (solid, red) in phosphate buffer saturated with O_2, and graphene/AuNPs/chitosan-modified electrodes in phosphate buffer saturated with N_2 (dotted, black) (Reprinted with permission from [53]).

exhibits longer shelf stability, as it does not contain any biomolecule within the matrix.

Zhao et al. [47] demonstrated that it is possible to prepare chitosan-dispersed graphene nanoflakes, which are dispersible in water, to form a stable, black aqueous solution. The prepared graphene nanoflakes were successfully immobilized on a GCE to construct a graphene-modified electrode. Cytochrome c was adsorbed on the surface of the modified electrode and the direct electron transfer of cytochrome c was observed. The authors claimed that cytochrome c on the surface of the electrode maintained its bioactivity and exhibited enzyme-like activity for the reduction of nitric oxide, presenting a potential application for the fabrication of novel biosensors to sense nitric oxide.

SGNFs were used for the enzymatic detection of glucose. Chaniotakis et al. [48] used SGNFs for the direct immobilization of enzymes onto a nanofiber surface (see Fig. 7). Surface modification of SGNFs with a biorecognition element was shown to be a highly efficient method for developing a new class of very sensitive, stable, reproducible electrochemical biosensors. Their results suggest that platelet nanofibers are the best materials so far described for developing biosensors, far superior to CNTs or graphite powder, as we also discussed in a work [27] mentioned above.

4.3. Graphene-supported nanoparticles for electrochemical detection

Graphene can be used as a conductive support for the deposition of electrocatalytic NPs [49]. Electrochemistry can be used to deposit catalytic NPs on graphene sheets [50]. Palladium NPs about 7–9 nm in diameter can be electrochemically deposited on defects on graphene sheets because of the preferred nucleation of the palladium at vacancies along the edges of intact, nanometer-sized graphene islands (see Fig. 8). The electrochemical route for the synthesis of the catalyst NPs is very attractive because the NPs nucleate at electroactive sites of carbon nanomaterials.

There are several excellent examples of applications of graphene decorated with catalyst NPs for electrochemical sensing and detection. Graphene was prepared by Hummer's chemical method and utilized as a catalyst support of Pt-Ru NPs for the electro-oxidation of methanol [51].

In another example, Li et al. [52] used gold NP (AuNP)-modified graphene for enzymatic detection of graphene. UV-visible absorption spectra indicated that GOx was biologically active even in the graphene-AuNP biocomposite. To construct a glucose biosensor, graphene, Au, and GOx were co-immobilized in Nafion to modify a GCE further. According to TEM and X-ray diffraction observation, the graphene sheets used in this work were single layer, or only a few layers. A similar graphene/AuNP/GOx nanocomposite was fabricated by Ramaprabhu et al. [53]. The authors claimed that the AuNPs prevented the graphene sheets from restacking.

Niu et al. constructed graphene/AuNP/chitosan nanocomposite electrodes (Fig. 9) [54]. Such electrodes demonstrated high electrocatalytic activity toward H_2O_2 and O_2. The authors suggested that the synergistic effect of graphene and the AuNPs might promote electrocatalysis toward H_2O_2. The high sensitivity and good stability of such a modified electrode contributed to the construction of a practical glucose biosensor.

5. Conclusions

It is clear that we have witnessed explosive growth in work related to the use of graphene-based electrodes for electrochemical sensing and biosensing. Some of the work is high quality and, in addition to interesting electrochemistry and sensing properties, it also contains detailed characterizations of graphene-related nanomaterials. However, some articles lack such characterization. We emphasize here that the output of graphene fabrication can differ significantly, even if there is only a slight variation in the method of preparation, so it is always necessary to provide detailed characterization data in order to avoid potential misinterpretation.

It is important to emphasize that graphene is a biocompatible nanomaterial [55], and, while serious toxicological effects were found with CNTs (mainly due to presence of metallic impurities within them [56]), graphene is non-toxic material. Another very important feature of graphene is the source materials. Graphene is mainly fabricated from graphite, which is inexpensive – while the opposite is true for CNTs, which are synthesized using NPs as templates from carbon-containing gases. In addition, graphene can provide more uniform and greater electroactive site distribution and density in order to decrease over-potentials, compared to graphite, and larger surface area (even larger than SWCNTs) for immobilization of biomolecules.

As we highlighted about the structure of ''graphenes'' in Section 1 above, all articles which use the term ''graphene'' referred to a multi-layered structure prepared from graphite. The morphology and the electrochemical performance of such multi-layer graphenes, prepared top down, differs significantly depending on their method of preparation. For the near future, we strongly advocate use of SGNFs prepared bottom up with more defined and controllable structures. In any case, detailed characterization of graphenes before using them in electrochemistry is essential if results are to have any meaning.

The use of nitrogen-doped and other heteroatom-doped graphene is of great interest, as such heteroatoms can provide electrocatalytic properties and enhance the stability of doped graphene electrodes.

We predict a bright future for graphene as a sensing material because of its biocompatibility, lack of metallic impurities (which are a major obstacle in electrochemical sensing research with CNTs), high conductivity and abundance of inexpensive source material. However, several major issues must be solved before truly single-sheet graphene can be used in analysis. First, restacking needs to be prevented by adding NPs. Very recently, a research-oriented solution of graphene single sheets appeared on the market [57]. In this case, the graphene is stabilized by surfactant. We should also mention here that there is no comparison of robustness of the graphene-based sensors in the literature. Clearly, analytical chemists have a great deal to do to address graphene-sensor behavior properly in the future.

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