Polyelectrolyte-graphene Nanocomposites for Biosensing Applications

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Abstract: Due to their unique structure, the optical and mechanical properties graphene and its derivatives (*e.g.* graphene oxide, reduced graphene oxide) have captured the attention of a constantly increasing number of scientists with regards to biomolecule sensing. This mini review focuses on one specific type of sensor, that consisting of graphene and polyelectrolytes. Polyelectrolyte-graphene nanocomposites exhibit outstanding detection capabilities by synergistically combining the characteristics of both components, outperforming traditional sensors in many cases. Characteristics and mechanistic details of the most important polyelectrolyte-graphene based sensors will be discussed in detail in addition to some current challenges and future perspectives.

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Keywords: Polyelectrolytes, graphene, nanocomposites, sensors, hybrid materials.

INTRODUCTION

Graphene is a newly utilized class of carbon-based materials as well as the building block for all graphitic forms and since its first production as an engineered material in 2004 [1] it has attracted a great deal of attention. The Nobel Prize in physics for experiments on graphene has certainly played a role in expanding the scope of research into new fields, including biomolecule sensing. This increased interest for graphene and its derivatives (*e.g.* graphene oxide (GO), reduced graphene oxide (rGO)) arises from its exceptional electrical, mechanical and thermal properties [2-4]. It is well established that the key material properties of graphene are associated with its structure, that of a single layer of two-dimensional sp^2 carbon atoms closely arranged into hexagonal cells.

Recently, significant attention has been given to the use of graphene and its derivatives in electrochemical, electronic and optical sensing applications [5]. The high surface area of graphene allows for a higher surface loading to target molecules for a given volume of graphene, while the excellent conductivity and small band gap are favorable for conducting electrons from the biomolecules [6]. Electron transfer can be enhanced because of the ability of graphene surfaces to support efficient electrical wiring between the electrode and the active redox centers of several biomolecules [7]. Furthermore, graphene-based sensors can exhibit a higher sensitivity since they exhibit low electronic noise from thermal effects. [8] Typically rGOs (see Fig. **1** for GO derivatives structures) are used in nanocomposites for sensing applications due to their comparative advantages over graphene and GO. These advantages include the reactive sites on rGO surfaces that allow convenient functionalization in contrast to graphene sheets, as well as the more efficient charge transport that ensures high electrochemical activity as compared to non-conductive GO. Recent studies have shown that the electrical conductivity of an original GO can be increased up to four orders of magnitude after reduction [9].

However, the superior properties of graphene are unique to the individual sheets and they do not apply to stacks or clusters of graphene sheets, making the prevention of aggregation an absolute necessity in order to take advantage of these properties for biosensing applications. Since aggregation occurs due to cohesive *van der Waals* interactions and π-π stacking, a disruption in these attractive forces can allow the graphene sheets to remain separated in a composite. Furthermore, to endow graphene with sensing capabilities it is often essential to functionalize it with recognition components that can assist in bringing detection targets onto the surface or in signal transduction. Additional advantages of graphene functionalization include increase in sensitivity, specificity, biocompatibility and loading capacity.

A promising way to achieve the above is through polymer functionalization, either by covalent attachment or through non-covalent interactions. For covalent attachment of polymers onto the graphene surface, polymers are either directly attached to graphene [10] or grown from its surface [11, 12] through reactive groups (*i.e.* oxygen, carboxylic, hydroxyl groups). These groups may already exist or may be introduced *via* chemical processing. Although covalent functionalization can lead to stable and effective nanocomposites, it inevitably alters the structure of the graphene by converting the sp^2 carbons to sp^3. This in turn causes a significant decrease in carrier mobility, reducing the potential of graphene in applications such as sensors. In contrast, non-covalent functionalization of graphene with polymers utilizes interactions such as π-π stacking [13], hydrophobic and electrostatic [14, 15], which do not change the structure of graphene and preserve its extraordinary properties.

In this mini-review, we will focus our attention on studies based on nanocomposites consisting of polyelectrolytes and graphene or its derivatives with attention to sensing applications. Compared to other types of polymers, polyelectrolytes are ideal candidates for non-covalent functionalization of graphene due to their highly charged nature. For example, cationic polyelectrolytes such as polyethyleneimine (PEI) can easily bind electrostatically with the negatively charged surface of GO inducing a net change in the graphene oxide surface charge, thus preventing aggregation [16] and through that increasing the suitability of graphene for sensing. Furthermore, the presence of charged groups in polyelectrolytes provides the opportunity for anchoring

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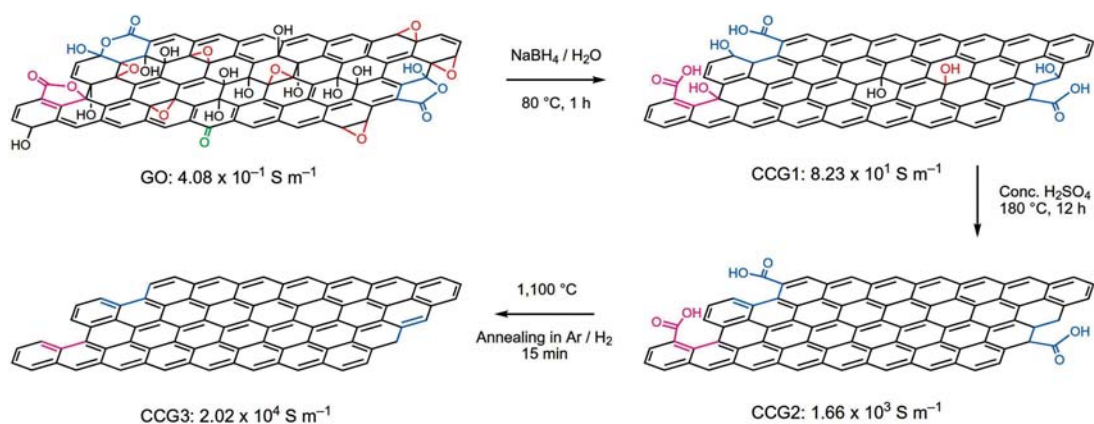
Fig. (1). Schematic representation of the GO reduction process. Go sheets are converted into other graphene-based materials by reduction and subsequent annealing (CCG represents the different levels of reduced graphene). Reprinted with permission from Macmillan Publishers Ltd: Nature Chemistry, 2009, 1, 403-408.
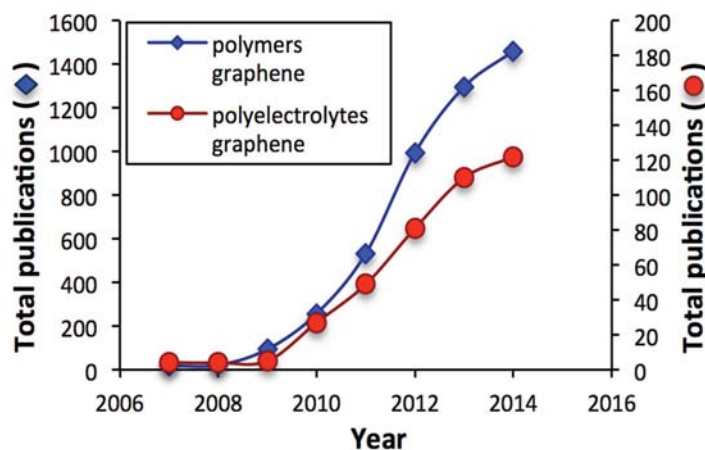
Fig. (2). Number of publications returned using "polymers" and "graphene" (left axes) or "polyelectrolytes" and "graphene" (right axes) as keywords in SciFinder (ISI-Web of Science and ScienceDirect showed similar results). Duplicates were removed.

nanoparticles, enzymes or other species through electrostatic interactions that are known in many cases to increase or assist sensing capabilities. Finally, graphene multilayers facilitate electron transfer enhancing the electrochemical reactivity.

Trends in the Field

Despite graphene's short history, the interest in polymer-graphene nanocomposites is clearly evident by the number of research publications (see Fig. **2**). Using "polymers" and "graphene" as keywords in any of the three most common databases (ISI-Web of Science, ScienceDirect, and SciFinder) a rapid increase in the total number of publications (from less than 10 in 2007 or before to more then 1,400 in 2014) can be seen. A similar trend in the number of publications is also observed using "polyelectrolyte" and "graphene" as keywords. This trend shows that the comparative advantage of the simultaneous use of polyelectrolytes and graphene is slowly being realized, especially if we take into account the wide variety of available polymers. Although polyelectrolyte-graphene nanocomposites have been used for a number of applications, our goal here is to present the latest developments on their use for the detection of biomolecules, including but not limited to detection of hydrogen peroxide, glucose, proteins and biomarkers.

DISCUSSION

Hydrogen Peroxide Detection

In the last few years, polyelectrolyte-graphene nanocomposites have been used as sensors for the detection of a number of molecules. One of the most frequently studied is hydrogen peroxide (H_2O_2), which is a product of many biological and environmental processes. In one of the earliest reports, Xu *et al*. [17] described a rGO-chitosan nanocomposite that immobilized a hemoglobin protein and exhibited bioelectrocatalytic activity toward H_2O_2. A schematic representation of the constructed sensor and the chemical structure of chitosan are presented in Figure **3**. The nanocomposite provides a favorable microenvironment for the protein to retain its native structure while the two dimensional rGO accelerates the electron transfer at the sensing interface. As a result, the polyelectrolyte-rGO sensor shows wide linear range (6.5-230 μM), low detection limit (0.51 μM) and good stability. Despite these impressive sensing abilities, enzymatic sensors cannot provide long-term stability due to the intrinsic nature of enzymes. Therefore, most polyelectrolyte-graphene nanocomposites for H_2O_2 sensing are actually based on enzyme-free strategies.
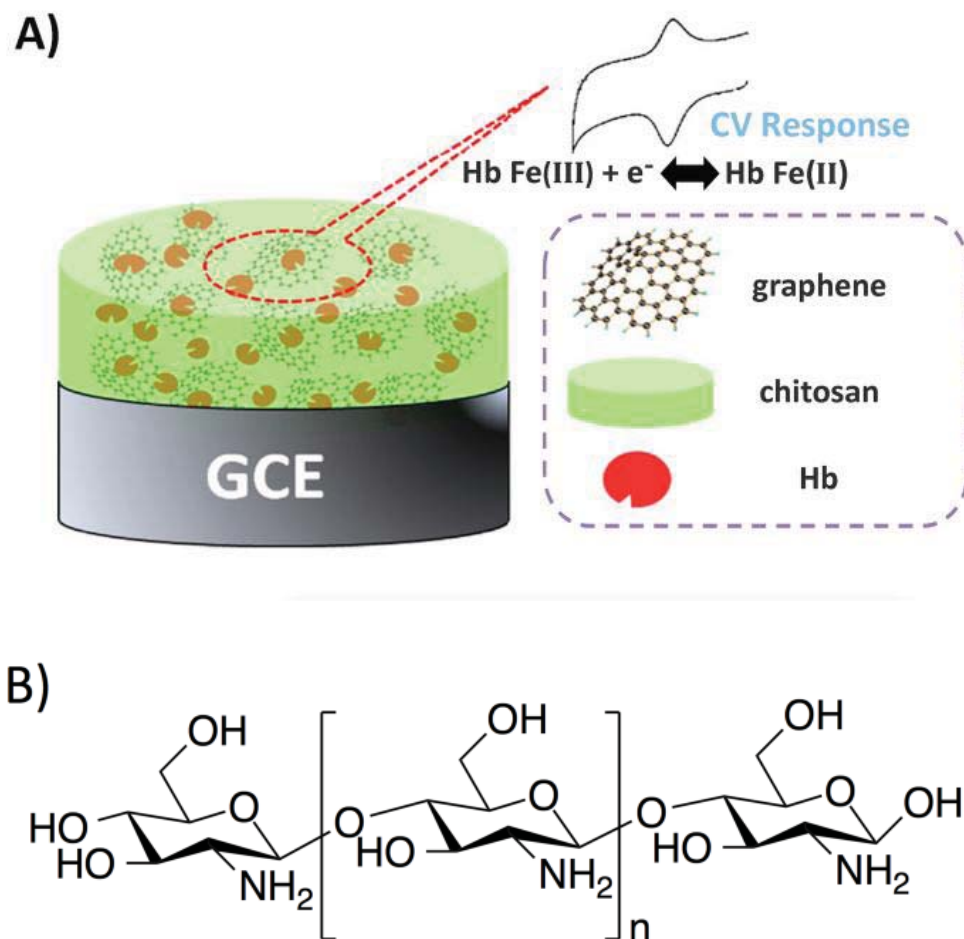
Fig. (3). A) Schematic representation of the hemoglobin-graphene-chitosan/Glassy Carbon Electrode (GCE) sensor and B) Chemical structure of chitosan. Reprinted from publication Talanta **2010**, 81, 334–338 (ref. 17), Copyright 2014, with permission from Elsevier.

Zeng *et al.* [18] used the Layer-by-Layer technique and combined different types of interactions to functionalize graphene sheets. Pyrene-grafted poly(acrylic acid) (PAA) was initially attached on a rGO surface through π-π stacking and *van der Waals* interactions, followed by attachment of PEI through electrostatic interactions. Graphene multilayer films (PEI/PAA-graphene) facilitated the electron transfer, enhancing the electrochemical reactivity of H_2O_2. The electrocatalytic activity can be tuned by altering the bilayer number of the graphene multilayer film $(PEI/PAA-graphene)_n$. The oxidation current value increases significantly in the initial two bilayers. However, the increase declines after three bilayers as the amount of newly adsorbed graphene sheets that can contact the electrode becomes smaller after the two bilayers are added. The $(PEI/PAA-graphene)_3$ electrode showed a rapid linear response to the change in H_2O_2 concentration (from 0.1 to 1 mM), reaching a steady state in only 5 s.

As mentioned earlier, polyelectrolytes can also serve as a method for the binding of catalytic nanoparticles and graphene due to their highly charged nature. The nanoparticles rough surfaces provide a higher electrochemically active area resulting in a higher turnover for heterogeneous catalytic or electrochemical reactions, which is why their use in polyelectrolyte-graphene sensors has been reported multiple times in the literature. For example, Fang *et al.* [19] demonstrated the use of cationic polyelectrolyte poly(diallyldimethyl ammonium chloride) (PDDA) functionalized graphene as the building block of graphene/Au nanoparticles hybrids (see Fig. **4**) for enhanced H_2O_2 sensing. The high-Au nanoparticle loaded nanocomposite showed a wide linear range (0.5μM-0.5mM) and lower detection limit (0.22 μM), compared to certain enzyme-based sensors. Very recently, a similarly PDDA functionalized GO decorated with platinum nanoparticles was prepared and used for the detection of H_2O_2 and methanol [20, 21]. A glassy carbon electrode modified with platinum nanoparticles-GO-PDDA was able to catalyze the reduction of H_2O_2 starting from 150 mV vs. Ag/AgCl while for methanol the ratio If/Ib (1.42) indicated that the catalyst has a good tolerance towards the intermediate carbonaceous species accumulated on the electrode surface.

A novel polyelectrolyte, poly[N-(1-one-butyric acid)benzimidazole] (PBI-BA) has also been used together with Au nanoparticles for the same purpose. The carboxylic groups and electroactive imine sites make PBI-BA a great candidate for use as an enzyme-free amberometric sensor for H_2O_2 [22]. Compared to other graphene based sensors, the thermostable (PBI-BA)/graphene/Au sensor offers a very rapid detection time (95% of steady-state current in 1.4 s after each addition of H_2O_2), a measurable linear range (2.5μM-5mM) and a relatively high sensitivity (1056 μA mM^{-1} cm^{-2}). Additionally it exhibits greater sensitivity, compared to the (PBI-BA)/Au electrode, highlighting the
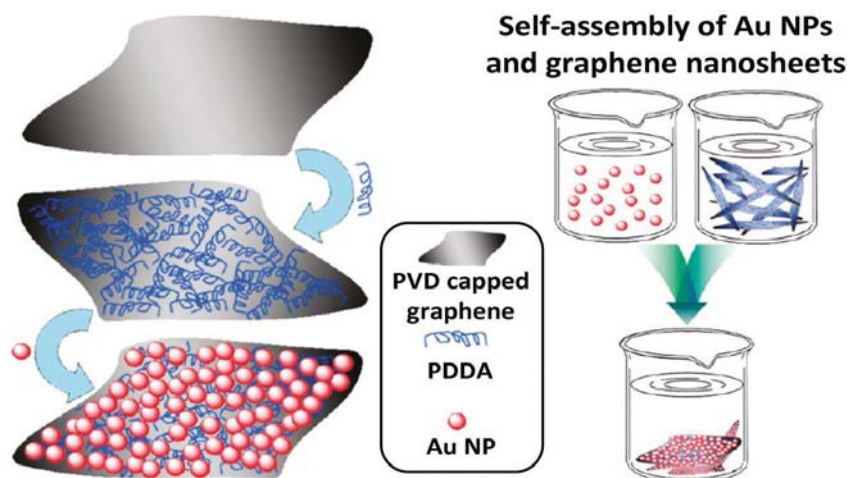
Fig. (4). Schematic representation of Au nanoparticles/PDDA-functionalized graphene nanosheets self-assembly. Reprinted with permission from Langmuir 2010, 26(13), 11277–11282. Copyright 2014 American Chemical Society.

importance of increased surface area and conductivity from graphene.

Prussian blue and its analogues are types of polynuclear transition-metal hexacyanometalates [23] that have been employed in electrochemical biosensors, due to their exceptional electrochromic and electroanalytical properties [24, 25]. The known ability of Prussian blue to reduce H_2O_2 at a very rapid catalytic rate with a low overpotential [26] is the main reason why a number of groups used it in polyelectrolyte-graphene nanocomposites to create an H_2O_2 sensor with varying results. [16, 27, 28] In these studies, PEI, PAA/PDDA and a cationic polyelectrolyte-functionalized ionic liquid [29] were used for the functionalization of graphene resulting in excellent dispersibility in water. In general Prussian blue/polyelectrolyte-graphene sensors exhibited quick sensing response [28] and linear ranges and sensitivities comparable to previously described studies.

Glucose, Dopamine and Guanosine Detection

Besides their use for H_2O_2, polyelectrolyte-graphene nanocomposites have been used for the detection of other important biomolecules such as glucose, dopamine and guanosine. In order to provide the desired dispersibility to the above polyelectrolyte-nanocomposite sensors chitosan has been used in almost every occasion for the functionalization of the graphene surface. Chitosan (see structure in Fig. **3B**) is a natural biopolymer with a unique combination of characteristics such as a primary amine at the C-2 position of the glucosamine residues, solubility in aqueous acidic media (pH lower than 6.5) and a biocompatible microenvironment for proteins or enzymes [30].

The electrochemical detection of glucose, which is very important for diabetes management, can be achieved with the use of glucose oxidase as the recognition element. Kang *et al.* [31] explored the direct electrochemistry of a chitosan-graphene/glucose oxidase nanocomposite. After adsorption on the graphene sheet, glucose oxidase retained its bioactivity and exhibited a fast heterogeneous electron transfer rate. The nanocomposite biosensor served as a glucose detector with a wide linearity (0.08 mM to 12 mM glucose), high sensitivity (37.93 A mM^{-1} cm^{-2}) and a detection limit of 0.02 mM, which is much lower than the diabetic glucose concentration (higher than 7.0 mM [32]).

Similar to the case of H_2O_2 sensors, nanoparticles have been used to decorate polyelectrolyte-graphene hybrid glucose sensors. For example, a one-step approach to prepare PDDA-graphene/SnO_2 nanocomposites (GSCN) was reported [33]. GSCN were produced by reducing GO/PDDA with Sn^{2+} ions in aqueous solution. Due to their positive charge the ions interact electrostatically with negatively charged metal nanoparticles creating ternary GSCN/nanoparticle hybrid structures. Prominent examples include Au and Pt nanoparticles loaded on the surface of GSCN. Au and Pt on GSCN at a high-density were used as the electrochemical material for non-enzymatic glucose detection with sufficient electrocatalytic activity (linearity 2 to 20 mM, sensitivity 20.3 µA mM^{-1}).

In addition to nanoparticle decoration and polymer functionalization, chemical doping with foreign atoms is another effective method to essentially modify the properties of graphene. Especially for carbonaceous materials, chemical doping can enhance free charge-carrier densities and electrical or thermal conductivities [34]. When compared to other dopants nitrogen is considered an ideal element for carbonaceous materials, due to its comparable atomic size and the five valence electrons available to form strong valence bonds with carbon atoms [35]. Furthermore, it has been demonstrated that N doping enhances the biocompatibility and sensitivity of carbonaceous structures (*e.g.* carbon nanotubes) in biosensing applications [36]. Inspired by this, Wang *et al.* reported a technique to synthesize N-doped graphene by treatment of graphene with nitrogen plasma and explored the potential of the doped graphene in electrocatalysis and biosensing applications [37]. The percentage of N in the graphene surface was controlled (varied from 0.11 to 1.35) through regulating the exposure time. N-doped graphene has exhibited great electrocatalytic activity for H_2O_2 reduction and fast direct electron transfer kinetics for glucose oxidase. An N-doped graphene-chitosan/glucose oxidase/glassy carbon electrode hybrid was successfully used for glucose biosensing (as low as 0.01 mM) in the presence of interferences.

Dopamine is an important neurotransmitter with a notable role in the central nervous, renal and endocrine systems functionality, while dopamine deficiency underlies Parkinson's diseases [38]. However, dopamine detection is challenging, as ascorbic acid is always coexisting with dopamine in organisms
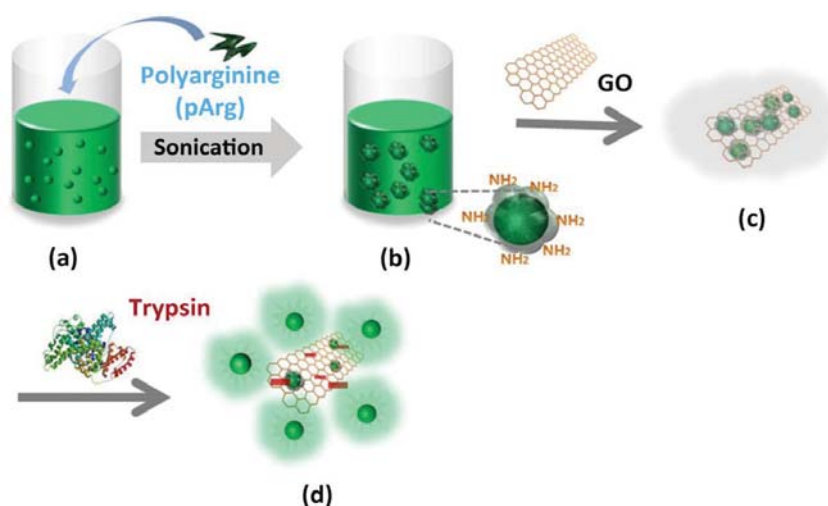
Fig. (5). Schematic illustration of trypsin detection using a polyelectrolyte-GO nanocomposite: (a) Green- emitting polymer conjugated nanoparticle, (b) Coating of nanoparticles with polyarginine, (c) Fluorescence-quenched *via* interaction of nanoparticles with GO, (d) Revival of green fluorescence *via* hydrolysis of polyarginine linker by enzymatic action of trypsin, leading to releasing of fluorescent nanoparticles. Reproduced from Ref. 50 with permission from John Wiley & Sons Inc. Copyright 2014.

and has a indistinguishable oxidation potential in electrochemical detection. A chitosan modified graphene electrode was developed by Wang *et al*. for the selective determination of dopamine concentration [39]. The linear range of the nanocomposite sensor was from 5 to 500 µM in the presence of large ascorbic acid excess (500 µM), which is an enhanced performance compared to multi-walled carbon nanotube-modified electrodes. In a different study by Li *et al*. [40], a Pt nanoparticle/polyelectrolyte-functionalized ionic liquid/graphene sheet nanocomposite was synthesized in one pot. The nanocomposite-modified electrode was able to determine ascorbic acid and dopamine based on a 200 mV difference between the peak potentials. The sensor was also successfully used in the analysis of human urine samples.

Nucleoside guanosine is a naturally endogenous compound with diverse biological activities including neuroprotective effects in cultured neurons and protection of brain slices subjected to hypoxia/hypoglycemia [41]. Additionally, changes of the guanosine concentration in human body have been used as indicators of a wide range of diseases (*e.g.* liver disease) [42]. Therefore, guanosine concentration determination is of increasing importance. A graphene, chitosan and Fe_3O_4 nanoparticle modified glassy carbon electrode was recently fabricated for the investigation of the electrochemical oxidation behavior of guanosine [43]. The presented results indicated that under optimized conditions the oxidation peak current is proportional to guanosine concentration in the range of 2.0×10^{-6} to 3.5×10^{-4} mol L^{-1} with a detection limit of 7.5×10^{-7} mol L^{-1} (S/N = 3). In addition, the proposed method showed the capability to distinguish the electrochemical oxidation response of guanosine, guanine and adenosine.

Biomarker, Enzyme and Protein Sensors

Peptide biomarkers are intriguing molecules that can serve as indicators of disease progression, bacterial infection and hormone levels, among many other abnormalities. However, peptide detection is still at an early stage [44-46]. In a recent work, Qin *et al*. [47] constructed an electrochemical aptasensor with high detection sensitivity and selectivity (detection concentration and range of 1 ng mL^{-1} and 1-265 ng mL^{-1}, respectively) to the D

enantiomer of arginine vasopressin peptide. The sensor was fabricated utilizing layer-by-layer assembly of methylene blue, PDDA and polystyrene sulfonate (PSS)-graphene layers. Graphene multilayers allow accumulation of methylene blue, which as mentioned before facilitates electron transfer. Most importantly, the multilayers provide adsorption sites for the molecular recognition agent (an aptamer). Aptamers are artificially selected single-stranded DNA/RNA oligonucleotides (between 40 and 100 bases) with a distinct three-dimensional structure. Due to their specific structure binding to specific target molecules can be achieved [48]. Differential pulse voltammetry experiments revealed that the aptasensor could serve as a sensing interface for detecting chiral peptides as well as other proteins such as lysozyme, bovine serum albumin, glutathione and α-thrombin.

In another biomarker study, Zhang *et al*. [49] used self-assembly of PDDA, PSS and graphene on polyethylene terephthalate to synthesize a non-labeled cancer marker biosensor for a real-time detection of a prostate specific antigen (PSA). For comparison a horseradish peroxidase (HRP) labeled sensor and a carbon nanotube based sensor were also prepared. The results showed the superiority of the non-labeled and labeled graphene sensors over the carbon nanotube one, with regards to detection limits. For the label free and labeled sensors, PSA concentrations down to 4 fg/ml and 4 pg/ml were detected, respectively. These concentrations are three orders of magnitude lower than the carbon nanotube based sensor. Different working mechanisms were described for the two types of biosensors. For the non-labeled case the conductance of the PSA modified biosensor monitors the changes of antibody with the concentration shifts of PSA solutions. In the labeled case, HRP-PSA antibody conjugates catalyze a biochemical reaction between ascorbic acid and H_2O_2. The local pH shifts allow the conductance variation of the biosensor.

In a different work, Noh *et al*. [50] fabricated a nanocomposite consisting of conjugated polymer nanoparticles and GO for the detection of trypsin (see schematic representation of sensor in Fig. **5**). Trypsin is an important digestive enzyme produced in the pancreas. The concentration of this particular enzyme in the human body has been related to a series of diseases [51] making
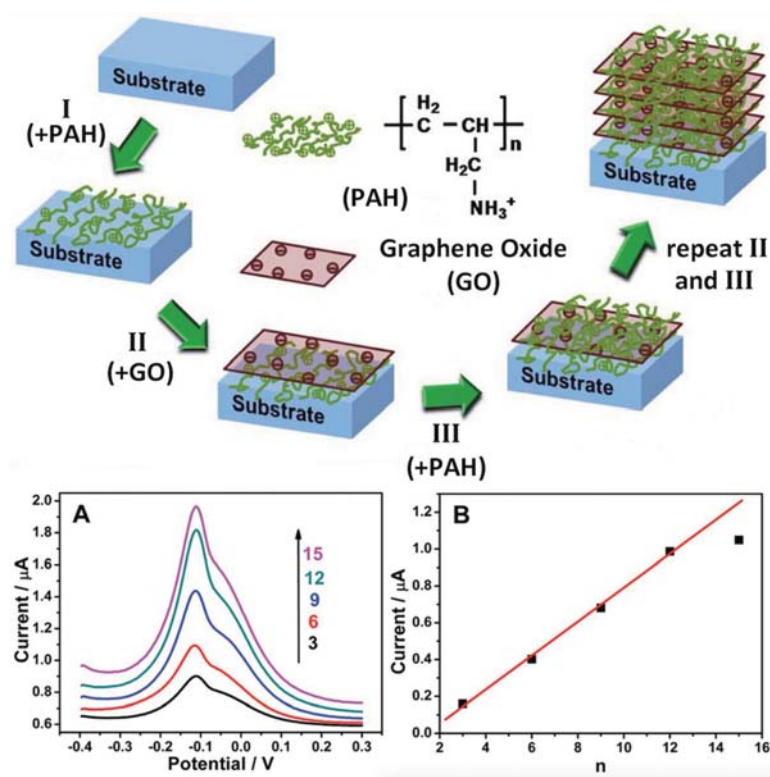
Fig. (6). Top: Schematic illustration of highly ordered nanocomposite electrodes of [PAH–GO]$_n$/glassy carbon electrode by layer-by-layer self-assembly. Bottom: A) Differential pulse anodic stripping voltammetry response of the [PAH–GS]$_{12}$-modified electrode towards the detection of Cu(II) and B) Plot of the peak currents vs. the concentration of Cu(II) ions. Reproduced from Ref. [15] with permission from The Royal Society of Chemistry.

its detection of great interest. Similarly to previous examples, the nanocomposite was synthesized using electrostatic attraction between positively charged fluorescent-conjugated polymer nanoparticles whose surface was modified with polyarginine and negatively charged GO. The key to the detection of trypsin is the quenching of the fluorescence of the conjugated nanoparticles by GO upon formation of the nanoparticle-polyarginine/GO nanocomposite. The fluorescence quenching is attributed to charge transfer interactions between the conjugated nanoparticles and the GO. Addition of the enzyme leads to selective degradation of polyarginine that serves as a linker between the nanoparticles and GO, resulting in the release of the nanoparticles. Therefore, the quenched fluorescence is recovered based on the concentration of trypsin (detection limit 0.827 µg/ml, concentration range 0-25 µg/ml). Since polyarginine is only degraded by trypsin, selectivity over other proteins can be achieved.

A technique that is similar in principal has been used for DNA detection using GO and a cationic conjugated polymer (poly [(9,9-bis(6-N,N,N-trimethylammonium)hexyl)-fluorenylene-phenylene dibromide], PFP) [52]. As in the case above, the key is the quenching of the fluorescent intensity of a fluorescein-labeled single stranded DNA (P) after the addition of GO. The fluorescence change of the P-GO-PFP nanocomposite appears to depend on the order of PFP and P addition. When PFP is added to P-GO the fluorescence resonance energy transfer (FRET) is insufficient, implying that the addition of PFP cannot release the P from the GO. On the other hand, when P is added to PFP-GO, efficient FRET is obtained attributed to the equal binding ability for P and PFP to GO [53]. Based on this phenomenon, a low background and amplified

fluorescent detection of DNA (as low as 40 pM of target DNA) could be easily achieved by measuring the change in fluorescence signal. The enhanced sensitivity (more than an order of magnitude lower than DNA detection using PFP) was attributed to the light harvesting properties of the PFP and the minimized background signal introduced by the addition of GO.

Humidity, Metal Ion and Nitrophenol Sensors

Detection of very low humidity levels is becoming increasing important, as even a small amount of water can have a large influence on a number of applications or procedures such as drying of medicine and gases, production of lithium-ion batteries, supercapacitors and many others. Despite the numerous efforts that include polyelectrolyte-based [54] or carbon nanotube/polyelectrolyte nanocomposites [55], satisfactory detection levels have not been reached yet. Li *et al.* [56] constructed an impedance-type humidity sensor *via* solution mixing of GO with PDDA or PSS polyelectrolytes and subsequent *in situ* reduction. In the absence of water molecules the two polyelectrolytes showed poor conductivity due to the limited ion conduction of the polyelectrolytes and could not be used to detect humidity lower than 10% relative humidity (HR) [57, 58]. The introduction of a highly conductive graphene surface enhanced the conductivity of the composite under dry atmosphere, thus allowing low humidity detection. The nanocomposites with higher graphene content exhibited lower impedance at low humidity levels (as low as 0.2% HR) and showed good response magnitude and relatively fast response times. The potential of polyelectrolyte-graphene nanocomposites for humidity sensing was revealed a few years earlier by the work of Zhang *et al.* [59] In this study

the electrical conductivity of the rGO/poly(N-vinyl pyrrolidone) (PVP) was measured at different levels of relative humidity using a conductive atomic force microscopy system and displayed a sensitivity to relative humidity variation with a response time of a few seconds.

Nitrophenols and their derivatives are major pollutants in industrial waste due to their extensive use as raw materials in chemical processing and their mutagenic, cyto and phyto-toxic effects. Out of the many nitrophenols 4-Nitrophenol is probably the most ubiquitous, which is why it is included in the US list of priority pollutants with a maximum permissible limit of less than 20 ppb. Peng *et al*. developed an electrochemical sensor based on PDDA-functionalized graphene for the detection of 4-Nitrophenol [60]. By optimizing experimental parameters such as the pH of the buffer solution, scan rate, accumulation time and potential, the PDDA/graphene/glassy carbon electrode sensor showed remarkable sensitivity and selectivity for 4-Nitrophenol detection (concentration range of 0.06-110 µM with a detection limit of 0.02 µM S/N = 3). Furthermore, the sensor showed adequate stability with sufficient reproducibility, accuracy and efficient 4-NP detection in aqueous samples.

In a very recent study, Liu *et al*. described how NH_2-rich graphene-polyelectrolyte nanocomposites can be used for the detection of trace Cu(II) [15]. The nanocomposite was prepared by alternating exfoliated GO species and polyallylamine hydrochloride (PAH) on a glassy carbon surface (see Fig. **6**), *via* a combination of electrostatic and hydrophobic interactions. This noncovalent functionalization method provides average coverage of the surface and appropriate distance of the available –NH_2. This is desirable for capture and release of the metal ions in solution. Additionally, the layered nanoscructure promotes electron transfer along the multilayer functional nanocomposite of [PAH–GS]$_{12}$. The performance of a (PAH-GO)$_{12}$/glassy carbon electrode towards Cu(II) detection in aqueous solution was evaluated with the use of differential pulse anodic stripping voltammetry with good results, as indicated by the linear dynamic range (0.5 to 50 mM for Cu(II)), low detection limit (0.35 mM) and long term stability.

CONCLUSIONS AND OUTLOOK

In this mini-review we summarize the recent developments in the use of polyelectrolyte-graphene nanocomposites as biological and chemical sensors. Out of the many available sensor designs, polyelectrolyte-graphene based sensors have specific comparative advantages. These are attributed primarily to the unique structural characteristics of graphene (*e.g.* high surface area, excellent conductivity and small band gap) and the charged nature of polyelectrolytes. The former increase surface loading of target molecules and favor electron transfer while the latter increase dispersibility of graphene without interrupting its structure and allow anchoring of species that increase/assist sensing capabilities. When taking into account the variety of graphene types (e,g. graphene, GO, rGO) and the large pool of polyelectrolytes available, the diversity of sensors reported to date does not come as a surprise. However, specific polyelectrolytes or graphene types such as chitosan, PDDA, and rGO are encountered more often, due to their special characteristics that include biocompatibility and more efficient charge transport.

With a constantly increasing variety of high efficiency sensors it becomes important to examine the challenges and limitations of these devices. One major concern is with the lack of

methods that would allow facile preparation of graphene and its derivatives with controlled characteristics (*e.g.* size) on a much bigger scale. Since the sensor performance is directly related to the surface area of the graphene, one can easily see how controlling the size is of great importance. Furthermore, variations in graphene size combined with the nature of the surface coating have been known to play a critical role in regulating the biological behavior and toxicity of graphene. Finally, a comprehensive understanding of the interactions between graphene, polyelectrolytes and target molecules, but also of the detection mechanisms still remains relevant. Although in most of the examples described here such understanding is sufficient to allow high performance sensing, as sensing systems become more and more complicated increasing the level of understanding is critical and will be absolutely necessary in order to sense multiple molecules simultaneously or detect single molecules. The above challenges could be the reason why the number of patents on polyelectrolyte-graphene sensors still remains low (less than 10 per year), compared to the increasing number of publications as shown in Figure **2**.

Because of the wide spectrum of polyelectrolyte-graphene nanocomposites, sensing areas are constantly expanding from the sensing of traditional biomolecules such as glucose or dopamine to new materials such as heavy metals or nitrophenols. These nanocomposite sensors exhibit excellent performance with regards to sensitivity, selectivity, response time and detection range, outperforming in many cases the state-of-the-art techniques. In numerous occasions this high performance is achieved through the incorporation of nanoparticles (*e.g.* Au, Fe_3O_4) and proteins (*e.g.* hemoglobin for H_2O_2 detection) in the nanocomposite layers or by graphene surface doping (*e.g.* N doping), showing that the potential of nanocomposites does not end with the choice of polyelectrolyte or graphene type. Despite the high performance that has already been achieved, unquestionably the full potential of polyelectrolyte-graphene or enriched polyelectrolyte-graphene nanocomposites has not yet been reached. The breadth of applications would certainly benefit from synergistic efforts of scientists with different backgrounds including chemistry, materials science, and biology. Such efforts could lead to the design of new polyelectrolytes for graphene functionalization or enhanced understanding of the sensing mechanisms and ultimately to the development of an even wider spectrum of excellent sensors.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

REFERENCES

[1]   Novoselov, K.S.; Geim, A.K.; Morozov, S.V.; Jiang, D.; Zhang, Y.; Dubonos, S.V.; Grigorieva, I.V.; Firsov, A.A. Electric field effect in atomically thin carbon films. *Science*, **2004**, *306*, 666-669.
[2]   Novoselov, K.S.; Geim, A.K.; Morozov, S.V.; Jiang, D.; Katsnelson, M.I.; Grigorieva, I.V.; Dubonos, S.V.; Firsov, A.A. Two-dimensional gas of massless dirac fermions in graphene. *Nature*, **2005**, *438*, 197-200.
[3]   Bunch, J.S.; van der Zande, A.M.; Verbridge, S.S.; Frank, I.W.; Tanenbaum, D.M.; Parpia, J.M.; Craighead, H.G.; McEuen, P.L. Electromechanical resonators from graphene sheets. *Science*, **2007**, *315*, 490-493.

[4] Balandin, A.A.; Ghosh, S.; Bao, W.; Calizo, I.; Teweldebrhan, D.; Miao, F.; Lau, C.N. Superior thermal conductivity of single-layer graphene. *Nano Lett.*, **2008**, *8*, 902-907.

[5] Liu, Y.; Dong, X.; Chen, P. Biological and chemical sensors based on graphene materials. *Chem. Soc. Rev.*, **2012**, *41*, 2283-2307.

[6] Stankovich, S.; Dikin, D.A.; Dommett, G.H.B.; Kohlhaas, K.M.; Zimney, E.J.; Stach, E.A.; Piner, R.D.; Nguyen, S.T.; Ruoff, R.S. Graphene-based composite materials. *Nature*, **2006**, *442*, 282-286.

[7] Zuo, X.; He, S.; Li, D.; Peng, C.; Huang, Q.; Song, S.; Fan, C. Graphene oxide-facilitated electron transfer of metalloproteins at electrode surfaces. *Langmuir*, **2010**, *26*, 1936-1939.

[8] Ao, Z.M.; Yang, J.; Li, S.; Jiang, Q. Enhancement of CO detection in Al doped graphene. *Chem. Phys. Lett.*, **2008**, *461*, 276-279.

[9] Gao, W.; Alemany, L.B.; Ci, L.; Ajayan, M. New insights into the structure and reduction of graphite oxide. *Nat. Chem.*, **2009**, *1*, 403-409.

[10] Liu, Z.; Robinson, J.T.; Sun, X.; Dai, H. PEGylated nanographene oxide for delivery of water- insoluble cancer drugs. *J. Am. Chem. Soc.*, **2008**, *130*, 10876-10877.

[11] Yang, Y.; Wang, J.; Zhang, J.; Liu, J.; Yang, X.; Zhao, H. Exfoliated graphite oxide decorated by PDMAEMA chains and polymer particles. *Langmuir*, **2009**, *25*, 11808-11814.

[12] Lee, S.H.; Dreyer, D.R.; An, J.; Velamakanni, A.; Piner, R.D.; Park, S.; Zhu, Y.; Kim, S.O.; Bielawski, C.W.; Ruoff, R.S. Polymer brushes *via* controlled, surface-initiated atom transfer radical polymerization (ATRP) from graphene oxide. *Macromol. Rapid Commun.*, **2010**, *31*, 281-288.

[13] Liu, J.; Tao, L.; Yang, W.; Li, D.; Boyer, C.; Wuhrer, R.; Braet, F.; Davis, T.P. Synthesis, characterization, and multilayer assembly of pH sensitive graphene–polymer nanocomposites. *Langmuir*, **2010**, *26*, 10068-10075.

[14] Hong, J.; Shah, N.J.; Drake, A.C.; DeMuth, P.C.; Lee, J.B.; Chen, J.; Hammond, P.T. Graphene multilayers as gates for multi-week sequential release of proteins from surfaces. *ACS Nano*, **2012**, *6*, 81-88.

[15] Liu, H.; Li, S.; Sun, D.; Chen, Y.; Zhou, Y.; Lu, T. Layered graphene nanostructures functionalized with NH_2-rich polyelectrolytes through self-assembly: Construction and their application in trace Cu(II) detection. *J. Mater. Chem. B*, **2014**, *2*, 2212-2219.

[16] Shan, C.; Wang, L.; Han, D.; Li, F.; Zhang, Q.; Zhang, X.; Niu, L. Polyethyleneimine- functionalized graphene and its layer-by-layer assembly with prussian blue. *Thin Solid Films*, **2013**, *534*, 572-576.

[17] Xu, H.; Dai, H.; Chen, G. Direct electrochemistry and electrocatalysis of hemoglobin protein entrapped in graphene and chitosan composite film. *Talanta*, **2010**, *81*, 334-338.

[18] Zeng, G.; Xing, Y.; Gao, J.; Wang, Z.; Zhang, X. Unconventional layer-by-layer assembly of graphene multilayer films for enzyme-based glucose and maltose biosensing. *Langmuir*, **2010**, *26*, 15022-15026.

[19] Fang, Y.; Guo, S.; Zhu, C.; Zhai, Y.; Wang, E. Self-assembly of cationic polyelectrolyte- functionalized graphene nanosheets and gold nanoparticles: A two-dimensional heterostructure for hydrogen peroxide sensing. *Langmuir*, **2010**, *26*, 7614-7618.

[20] Bragaru, A.; Vasile, E.; Obreja, C.; Kusko, M.; Danila, M.; Radoi, A. Pt nanoparticles on graphene – polyelectrolyte nanocomposite: Investigation of H2O2 and methanol electrocatalysis. *Mater. Chem. Phys.*, **2014**, *146*, 538-544.

[21] Liu, H.; Song, C.; Zhang, L.; Zhang, J.; Wang, H.; Wilkinson, D. P. A review of anode catalysis in the direct methanol fuel cell. *J. Power Sour.*, **2006**, *155*, 95-110.

[22] Hua, M.Y.; Chen, H.C.; Tsai, R.Y.; Leu, Y.L.; Liu, Y.C.; Lai, J.T. Synthesis and characterization of carboxylated polybenzimidazole and its use as a highly sensitive and selective enzyme-free H_2O_2 sensor. *J. Mater. Chem.*, **2011**, *21*, 7254-7262.

[23] Ellis, D.; Eckhoff, M.; Neff, D. Electrochromism in the mixed-valence hexacyanides. 1. Voltammetric and spectral studies of the oxidation and reduction of thin films of prussian blue. *J. Phys. Chem.*, **1981**, *85*, 1225-1231.

[24] Li, J.; Qiu, J.D.; Xu, J.J.; Chen, H.Y.; Xia, X.H. The synergistic effect of prussian-blue- grafted carbon nanotube/poly(4-vinylpyridine) composites for amperometric sensing. *Adv. Funct. Mater.*, **2007**, *17*, 1574-1580.

[25] Karyakin, A.A.; Karyakina, E.E.; Gorton, L. Amperometric biosensor for glutamate using prussian blue-based "artificial peroxidase" as a transducer for hydrogen peroxide. *Anal. Chem.*, **2000**, *72*, 1720-1723.

[26] Moscone, D.; D'Ottavi, D.; Compagnone, D.; Palleschi, G.; Amine, A. Construction and analytical characterization of prussian blue-based carbon paste electrodes and their assembly as oxidase enzyme sensors. *Anal. Chem.*, **2001**, *73*, 2529-2535.

[27] Mao, Y.; Bao, Y.; Wang, W.; Li, Z.; Li, F.; Niu, L. Layer-by-layer assembled multilayer of graphene/prussian blue toward simultaneous electrochemical and SPR detection of H_2O_2. *Talanta*, **2011**, *85*, 2106-2112.

[28] Ma, J.; Cai, P.; Qi, W.; Kong, D.; Wang, H. The layer-by-layer assembly of polyelectrolyte functionalized graphene sheets: A potential tool for biosensing. *Colloid. Surf. A: Physicochem. Eng. Aspects*, **2013**, *426*, 6-11.

[29] Shen, Y.; Zhang, Y.; Zhang, Q.; Niu, L.; You, T.; Ivaska, A. Immobilization of ionic liquid with polyelectrolyte as carrier. *Chem. Commun.*, **2005**, (33), 4193-4195.

[30] Zhang, M.; Smith, A.; Gorski, W. Carbon nanotube−chitosan system for electrochemical sensing based on dehydrogenase enzymes. *Anal. Chem.*, **2004**, *76*, 5045-5050.

[31] Kang, X.; Wang, J.; Wu, H.; Aksay, I. A.; Liu, J.; Lin, Y. Glucose oxidase–graphene–chitosan modified electrode for direct electrochemistry and glucose sensing. *Biosens. Bioelectron.*, **2009**, *25*, 901-905.

[32] Dai, Z.H.; Ni, J.; Huang, X.H.; Lu, G.F.; Bao, J.C. Direct electrochemistry of glucose oxidase immobilized on a hexagonal mesoporous silica-MCM-41 matrix. *Bioelectrochemistry*, **2007**, *70*, 250-256.

[33] Zhu, C.; Fang, Y.; Wen, D.; Dong, S. One-pot synthesis of functional two-dimensional graphene/SnO_2 composite nanosheets as a building block for self-assembly and an enhancing nanomaterial for biosensing. *J. Mater. Chem.*, **2011**, *21*, 16911-16917.

[34] Zhou, C. Modulated chemical doping of individual carbon nanotubes. *Science*, **2000**, *290*, 1552-1555.

[35] Lee, S.U.; Belosludov, R.V.; Mizuseki, H.; Kawazoe, Y. Designing nanogadgetry for nanoelectronic devices with nitrogen-doped capped carbon nanotubes. *Small*, **2009**, *5*, 1769-1775.

[36] Carrero-Sánchez, J.C.; Elías, A.L.; Mancilla, R.; Arrellín, G.; Terrones, H.; Laclette, J.P.; Terrones, M. Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen. *Nano Lett.*, **2006**, *6*, 1609-1616.

[37] Wang, Y.; Shao, Y.; Matson, D. W.; Li, J.; Lin, Y. Nitrogen-doped graphene and its application in electrochemical biosensing. *ACS Nano*, **2010**, *4*, 1790-1798.

[38] Heien, M.L.A.V.; Khan, A.S.; Ariansen, J.L.; Cheer, J.F.; Phillips, P.E.M.; Wassum, K.M.; Wightman, R.M. Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 10023-10028.

[39] Wang, Y.; Li, Y.; Tang, L.; Lu, J.; Li, J. Application of graphene-modified electrode for selective detection of dopamine. *Electrochem. Commun.*, **2009**, *11*, 889-892.

[40] Li, F.; Chai, J.; Yang, H.; Han, D.; Niu, L. Synthesis of Pt/ionic liquid/graphene nanocomposite and its simultaneous determination of ascorbic acid and dopamine. *Talanta*, **2010**, *81*, 1063-1068.

[41] Frizzo, M.E.D.S.; Lara, D.R.; Prokopiuk, A.S.; Vargas, C.R.; Salbego, C.G.; Wajner, M.; Souza, D.O. Guanosine enhances glutamate uptake in brain cortical slices at normal and excitotoxic conditions. *Cell. Mol. Neurobiol.*, **2002**, *22*, 353-363.

[42] Goyal, R.N.; Gupta, V.K.; Oyama, M.; Bachheti, N. Voltammetric determination of adenosine and guanosine using fullerene-C60-modified glassy carbon electrode. *Talanta*, **2007**, *71*, 1110-1117.

[43] Yin, H.; Zhou, Y.; Ma, Q.; Ai, S.; Chen, Q.; Zhu, L. Electrocatalytic oxidation behavior of guanosine at graphene, chitosan and Fe_3O_4 nanoparticles modified glassy carbon electrode and its determination. *Talanta*, **2010**, *82*, 1193-1199.

[44] Ravindranath, S.P.; Mauer, L.J.; Deb-Roy, C.; Irudayaraj, J. Biofunctionalized magnetic nanoparticle integrated mid-infrared pathogen sensor for food matrixes. *Anal. Chem.*, **2009**, *81*, 2840-2846.

[45] Turney, K.; Drake, T.J.; Smith, J.E.; Tan, W.; Harrison, W.W. Functionalized nanoparticles for liquid atmospheric pressure matrix-assisted laser desorption/ionization peptide analysis. *Rapid Commun. Mass Spectrom.*, **2004**, *18*, 2367-2374.

[46] Tamvakopoulos, C. Mass spectrometry for the quantification of bioactive peptides in biological fluids. *Mass Spectrom. Rev.*, **2007**, *26*, 389-402.

[47] Qin, H.; Liu, J.; Chen, C.; Wang, J.; Wang, E. An electrochemical aptasensor for chiral peptide detection using layer-by-layer assembly of polyelectrolyte-methylene blue/polyelectrolyte- graphene multilayer. *Anal. Chim. Acta*, **2012**, *712*, 127-131.

[48] Mir, M.; Vreeke, M.; Katakis, I. Different strategies to develop an electrochemical thrombin aptasensor. *Electrochem. Commun.*, **2006**, *8*, 505-511.

[49] Zhang, B.; Cui, T. An ultrasensitive and low-cost graphene sensor based on layer-by-layer nano self-assembly. *Appl. Phys. Lett.*, **2011**, *98*, 073116.

[50] Noh, J.; Chae, B.J.; Ku, B.C.; Lee, T.S. Fabrication of a nanohybrid of conjugated polymer nanoparticles and graphene oxide for biosensing of trypsin. *J. Polym. Sci. A Polym. Chem.*, **2014**, *52*, 1898-1904.

[51] Yamashita, K.; Mimori, K.; Inoue, H.; Mori, M.; Sidransky, D. A tumor-suppressive role for trypsin in human cancer progression. *Cancer Res.*, **2003**, *63*, 6575-6578.

[52] Xing, X.J.; Liu, X.G.; He, Y.; Lin, Y.; Zhang, C.L.; Tang, H.W.; Pang, D.W. Amplified fluorescent sensing of DNA using graphene oxide and a conjugated cationic polymer. *Biomacromolecules*, **2013**, *14*, 117-123.

[53] Gaylord, B.S.; Heeger, A.J.; Bazan, G.C. DNA hybridization detection with water-soluble conjugated polymers and chromophore-labeled single-stranded DNA. *J. Am. Chem. Soc.*, **2003**, *125*, 896-900.

[54] Su, P.G.; Li, W.C.; Tseng, J.Y.; Ho, C.J. Fully transparent and flexible humidity sensors fabricated by layer-by-layer self-assembly of thin film of poly(2-acrylamido-2-methylpropane sulfonate) and its salt complex. *Sensor. Actuator. B*, **2011**, *153*, 29-36.

[55] Yoo, K.P.; Lim, L.T.; Min, N.K.; Lee, M.J.; Lee, C.J.; Park, C.W. Novel resistive-type humidity sensor based on multiwall carbon nanotube/polyimide composite films. *Sensor. Actuator. B*, **2010**, *145*, 120-125.

[56]   Li, Y.; Deng, C.; Yang, M. Facilely prepared composites of polyelectro-lytes and graphene as the sensing materials for the detection of very low humidity. *Sensor. Actuator. B*, **2014**, *194*, 51-58.

[57]   Rauen, K.L.; Smith, D.A.; Heineman, W.R. Humidity sensor based on conductivity measurements of a poly(dimethyldiallylammonium chloride) polymer film. *Sensor. Actuator. B*, **1993**, *17*, 61-68.

[58]   Li, Y.; Yang, M.J.; She, Y. Humidity sensors using in situ synthesized sodium oilystyrene sulfonate/ZnO nanocomposiyes. *Talanta*, **2004**, *62*, 707-712.

[59]   Zhang, J.; Shen, G.; Wang, W.; Zhou, X.; Guo, S. Individual nanocomposite sheets of chemically reduced graphene oxide and poly(n-vinyl pyrroli-done): Preparation and humidity sensing characteristics. *J. Mater. Chem.*, **2010**, *20*, 10824-10828.

[60]   Peng, D.; Zhang, J.; Qin, D.; Chen, J.; Shan, D.; Lu, X. An electrochemical sensor based on polyelectrolyte-functionalized graphene for detection of 4-nitrophenol. *J. Electroanal. Chem.*, **2014**, *734*, 1-6.