Electrochemical biosensing using N-GQDs: Recent advances in analytical approach

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ABSTRACT

This review is meant to provide an overview of the electrochemical biosensors based on Nitrogen doped graphene quantum dots (N-GQDs) for analytical approaches, along with significant advances over the last several years in related technologies. In addition, this review described: i) Most frequently applied principles in biosensing based on N-GQDs ii) The aspects of fabrication in the perspective of biosensing applications iii) The potential of various electrochemical, biosensors for the determination of target analytes within sub-micromolar range and the circumvention of the most serious problem in biosensing will be discussed. iv) Some of multiplex electrochemical biosensors have been discussed with and without labels. v) We also summarize the latest developments in the applications of biosensors methods for detection of important analytes in real samples. vi) The development trends of biosensors are also introduced, including newly developed integrated biosensors based on (N-GQDs).

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1. Introduction

1.1. Biosensing; principles of biosensor function

Biosensing are based on immobilized biomolecules for the detection and determination of target analytes. Such sensing biomolecules should be attached to the surface of a signal transducer. After interaction, the biological recognition event generates an optical or electrical signal. Thus, the biomolecule immobilization plays a crucial role in achieving with extended device lifetime. The substrate materials such as electrodes, mesoporous materials, nanoparticles (NPs), nanotubes, graphene, etc., for biomolecule immobilization must be modified to introduce functional groups that attached to biomolecules with high bonding strength, excellent long-term stability, biocompatibility, and high activity [1]. In general, a biosensor is an analytical device that is composed of two components, a bioreceptor and a transducer. First part, the bioreceptor is a biomolecule that recognizes the target analyte, and second part the transducer converts the recognition event into a measurable signal [2].

1.2. Electrochemical biosensing

Electrochemical biosensors combine the analytical power of electrochemical techniques with the specificity of biological recognition processes [3–7]. The basic principle of electrochemical biosensors is that chemical reaction between immobilized biomolecule and target analyte producing or consuming ions or electrons, which affects measurable electrical properties of the solution, such as electric current or potential. In bioelectrochemistry, the reaction would either generate a measurable current (amperometric), a measurable potential or charge accumulation (potentiometric) or measurably alter the impedance (both resistance and reactance) of a medium between electrodes [8].

1.3. Graphene quantum dots (GQDs)

In the recent years, two types of prominent carbon nanomaterial, graphene quantum dots (GQDs) and graphene oxide (GO), have drawn increasing attention in sensor application. GQDs are nanometer-sized fragments of graphene. GQDs are a type of 0D
material with features obtaining from both graphene and carbon dots (CDs), GQDs having extraordinary features associated with quantum confinement and edge effects. GQDs with sizes less than 10 nm are nanometer-sized fragments of graphene. GQDs have particular electrical, high biocompatibility, low toxicity, easy operation, excellent biocompatibility, moderate preparation conditions, biocompatibility, chemical inactiveness, inexpensive and wide source, and aqueous solubility. Physical and chemical properties which are known as the newest developed materials. Consequently, they propose as appropriate candidates for utilize in electrochemical sensors. Taking advantage of the electrochemical features identical to graphene, GQDs are extensively applied in numerous areas of optoelectronic tools, biosensing, bioimaging applications, photocatalysis, environmental, and biological applications [9].

1.4. Nitrogen-doped graphene quantum dots (N-GQDs)

Nitrogen is regarded to be an outstanding element for the chemical doping of GQDs ascribed to its matchable atomic size and five valence electrons for joining with carbon atoms [10]. Nitrogen doping has been a subsequent way to alter the features of carbon materials varying from activated carbon to graphene. Nitrogen substitution, or substantial N-doping, has been a subsequent procedure to alter graphitic carbon materials for several applications, varying from catalysis to microelectronics [10], N-doped activated carbon or carbon nanotube, for instance, possess catalytic activities for some significant chemical reactions such as oxygen decrease and dehydrochlorination. In graphene, substitution nitrogen atoms are able to sufficiently alter the electronic construction of the host, supplying an assurance way to tailor the transport features of graphene for electronic applications [10].

Nitrogen-doped graphene quantum dots (N-GQDs) are nitrogen-doped graphene mends (Fig. 1) that exhibit impressive electrocatalytic activities, zero-dimensional N-GQDs are scarcely indicated as one electrocatalyst in fuel cells [11]. The N-GQDs are favorably crystallizing with a size of 2.5–8.5 nm of diameter and made up 5–7 layers of thickness. N-GQDs was initially reported by Qu et al., in 2011 by a simple electrochemical approach [11].

To date, great attempts have been made in the preparation of N-GQDs from “top-down” and “bottom-up” approaches [12]. The top-down strategies indicate to producing massive carbon materials into small GQDs accompanied by nitrogen doping, consisting electrochemical [13], hydrothermal or nitrogen plasma manner [14]. Presently, these top-down approaches for the manufacture of N-GQDs suffer from low obtainments and troubles in monitoring the morphology and size distribution. The bottom-up techniques of unit cell assembly have advantages over the top-down ways on the tuning of composition, size, and physical features of N-GQDs by ranging the organic precursors and reaction circumstances [15].

N-GQDs present outstanding physicochemical features and display assurance applications in many areas. still, the synthesis techniques for N-GQDs involve the solution chemistry method, electrochemical method, organic method, acid oxidation [12–14], solvochemical preparation and hydrothermal method [12–14]. Between them, the hydrothermal method is extensively adopted on account of to its simple operation, moderate reaction circumstances and use of partly cheap device. At the same time, in order to prevent organic reagents or costly ionic liquid as a nitrogen source in the synthesis of N-GQDs, the selection of green and low-price nitrogen sources such as amino acids has become a significant survey direction [12–14].

1.5. General application of N-GQDs

Recent survey has presented that, researchers discovered that the features of GQDs could be extremely tuned by nitrogen-doping. The several nitrogen doped colloidal graphene quantum dots gained by the solution chemistry approach in their survey exhibited a size-reliant electrocatalytic function for the oxygen reduction reaction [10]. Despite the dramatic advance that has been made for electrochemically active N-GQDs towards the oxygen reduction reaction (ORR), the improved electrocatalytic activity is still confined, which may be associated to the low electrical conductivity of the electrode made by utilizing small GQDs with high percolation threshold values, a significant factor in assigning the electrocatalytic oxygen reduction reaction ORR function.

More recently, Fei et al., have displayed a technique, in which they first blend GQDs with graphene, a famous carbon support for electrocatalysts, and then co-dope the hybrid with boron and nitrogen to gain BN-doped GQD/graphene hybrid nanoplatelets. The out coming hybrid material has showed synergistic impacts to improve the ORR electrocatalytic activity [15]. Gong and co-workers. In addition, an efficient two-photon fluorescent N-GQD probe with low cytotoxicity and good photo-consistency for cellular imaging were prepared recently [16]. Researchers of this work illustrated that the nitrogen-doping onto GQDs with subsequent electron-donating dimethylamido groups resulted in a red shift of its fluorescent emission. The large π-conjugated graphene sheets and the subsequent electron donating impact of dimethylamido facilitated the charge transport efficiency and led to strong two-photoninduced fluorescence for N-GQD [17]. Chinese researchers stated that N-doped GQDs with O-rich functional groups exhibit great electrocatalytic activities [18–22].

Jiang et al., proved that N-GQDs could be utilized as an effective photocatalyst for photochemical synthesis of silver nanoparticle loaded porous graphitic C₃N₄ (Ag/p-g-C₃N₄). The prosperous and effective preparation of the Ag/p-g-C₃N₄ composite was due to the outstanding electron-donating capability of photo-excited N-GQD, which performs both as a photosensitizer and a decreasing agent because of its extensive visible light absorption under visible light [18]. Recently, Ju and Chen [22] developed a simple strategy to prepare N-GQDs and made a fluorescent sensing platform for Fe³⁺ discovery with an extensive focus range, pointing the promise for future applications of N-GQDs in the area of analysis (Fig. 2). In spite of that, as advanced electrode materials, N-GQDs-based electrochemical sensors have rarely been stated and so, it’s essential to develop new ways to prepare N-GQDs and expand the applications of the N-GQDs in the electroanalytical area [20].

This article reviews recent advances in the N-GQDs based electrochemical biosensors for detection of target analyte. It is important to point out that, due to the explosion of publications in this active field, we do not claim that this review includes all of the
published works in the past years and we apologize to the authors of excellent work, which is unintentionally left out. Therefore, with the selected latest research articles from 2013 to 2017; application of N-GQDs in biosensors for detection of target analytes in was discussed and summarized in this review. This review is meant to provide an overview of the various types of biosensors based on Nitrogen doped graphene quantum dots (N-GQDs) for analytical approaches, along with significant advances over the last several years in related technologies. In addition, this review described: i) Most frequently applied principles in biosensing based on of N-GQDs ii) The aspects of fabrication in the perspective of immunoassay/immunosensing applications iii) The potential of various electrochemical biosensors for the determination of target analytes within sub-micromolar range and the circumvention of the most serious problem in immunosensing/immunoassay will be discussed. iv) Some of multiplex electrochemical biosensors have been discussed with and without labels. v) We also summarize the latest developments in the applications of biosensors methods for detection of important analytes in real samples. vi) The development trends of electrochemical based biosensors are also introduced, including newly developed integrated biosensors, and the application of (N-GQDs).

This review covers the state of the art in electrochemical biosensors based on N-GQDs. The purpose of the review is to provide useful insights in terms of (a) choice of materials, (b) methods/techniques preferably applied, (c) detection procedures, (d) immobilization procedures, and (d) other practical aspects. Following an introduction into the field, we give a short account on the principle and general types of biosensors, and then treat biosensors for targeted analytes. This short review covers electrochemical biosensors, with subsections on using of N-GQDs in the structure of immune-, apta-, geno-, cyto-, enzyme, and non-enzymatic biosensors). The review also covers aspects of surface modifications, the effect of N-GQDs properties on sensing performance, and the applications of N-GQDs in conjunction with different kind of signal transducers.

2. Types of application

Chemical-doping technique displays a subsequent equipment for altering the electronic and chemical features of the host materials. It has already played an essential role in the survey of material chemistry and electrochemistry. Electrochemical techniques have many profits, such as high selectivity, low price, fast analysis, low detection limit, ease in use, and portability. The electrochemical activity of graphene can be improved by tuning its electronic construction, which can be gained by heteratoms doping and size monitoring. Nitrogen doping is regarded to be one of the most effective approaches to favorably tuning the features of graphene, like its electrocatalytic activity, chemical consistency, and electrical conductivity, etc. Doping of nitrogen into graphene (N-graphene), especially in the form of the pyridine moiety, can sufficiently improve the electrocatalytic activity for H2O2 reduction [21]. The theoretical calculation pointed that nitrogen doping results in subsequent and effective conjugation between the nitrogen lone-pair electrons and the graphene π—system, leading to modification of its spin density and charge distribution, which in turn manufactures an activated region on the graphene surface [21].
In the case of electrochemical biosensing based on N-GQDs, the use of these advanced nanomaterials for improvement of electrochemical performances and signal amplification was discussed. Also, we summarized below examples of N-GQDs applications in electrochemical immunosensing, aptasensing, geno-sensing and ion-sensing, with their advantages and limitations, and their potential for future development in this field was stressed. Therefore, major focus of this review is on the role of N-GQDs in biosensing. With the selected latest research articles from 2013 to November 2017, we summarized various electrochemical biosensing approaches for detection of target analytes in this section of review. More importantly, we discussed in detail different aspects such as type of nanomaterials conjugated with N-GQDs, injection and detection techniques, labels, analytes and the corresponding sample matrix, and sensitivity. Consequently, we discussed several outstanding properties of the electrochemical biosensing for detection of different analytes and their research opportunities as well as the development potential and prospects.

2.1. Immunosensor

Electrochemical immunosensor has obtained extensive attention because of its economical, easy-to-perform, portable, sensitive and plain to make features. As an essential area of electrochemical immunosensors, the label-free immunosensors have an advantage over sandwich-sort immunosensors because the sensor structure is simpler. In addition, the non-enzymatic label-free immunosensors have prevail some obstacles of enzyme-based biosensors, null immobilization series, such as environmental in consistency, and high price.

In order to enlarge the discovery signal, several sorts of materials is utilized to invent the immunosensors, consist of nanoparticles, metal oxides, quantum dots and electroactive component-encapsulated nanoparticles. Between the nanoparticles, the noble metal with well-defined, low-price and monitored shape have appealed high attention and become a sort of promising materials in ultrasensitive chemical and biological molecules detections. For instance, gold nanoparticles, palladium nanoparticles, metal nanocatalysts and platinum nanoparticles have been extensively used for the invention of electrochemical biosensors due to their well biocompatibility, outstanding electrocatalytic activity, and high electro conductive. Compared with individual metal nanoparticles, bimetallic nanoparticles compound the catalytic impact of their monometallic counterparts to produce particularly special properties. It is famous that, in electrocatalysis and electrochemical immunosensor applications, metal nanoparticles are usually distributed on supporting materials to gain high conductivity, consistancy and large surface.

Recently Yang and co-workers proposed a new and ultrasensitive label-free electrochemical immunosensor for quantitative discovery of carcinoembryonic antigen (CEA). In this work, the N-GQDs supported PtPd bimetallic nanoparticles (PtPd/N-GQDs) were combined by a plain and green hydrothermal process (Fig. 3). Afterward, PtPd/N-GQDs functionalized Au nanoparticles (PtPd/N-GQDs@Au) were prepared successfully via a self-assembly approach. Due to the synergetic impact display in PtPd/N-GQDs@Au, this new nano-composites has displayed outstanding electrocatalytic activity towards hydrogen peroxide ($H_2O_2$) reduction. PtPd/N-GQDs@Au was applied as transducing materials to efficiently combine capture antibodies and increase electrochemical signal. Under the optimum states, the suggested immunosensor was utilized for the discovery of CEA with extensive static range from 5 fg/mL to 50 ng/mL with a low detection limit of 0.2 fg/mL [21].

In summary, some of N-GQDs-based electrochemical immunosensors for targeted analytes are discussed in this sub-section. Importantly, the use of N-GQDs would most likely result in enhanced analytical characteristics of the electrochemical immunosensors, particularly with respect to operational stability, life-time, and operation in harsh conditions. The presence of diffusion barriers and possible antigen entrapping are major limitations, resulting in high response times and instability problems. Even though several strategies are available to avert entrapping, these are generally complex and poorly reproducible, involving multiple processing steps prior to antibody immobilization. Furthermore, their successful use in immunosensors is strongly dependent on how efficient the antigen-N-GQDs assembly is attracted on to the physical transducer surface and the homogeneity of the N-GQDs layer.

While reported results are exciting and exhibit great potential for future applications, new breakthroughs are still required for immunosensing. Although the fast advancement of electrochemical immunosensors highlights their future applications in diverse scientific fields, the major impacts of such technologies appear to be in early detection of cancer. Of these applications, early detection of cancer seems to provide an interesting approach for the development of novel target-based therapies (e.g., using electrochemical/photonic and photoelectrochemical probes for detection of target biomarkers).

As many of these immunosensors have been only developed in laboratory so far, it will be a major challenge to introduce a technology allowing rapid production of large numbers of sensors with relatively low cost and high quality specifications. Such technology is prerequist for any successful commercial application.

At the end of this review, it is worth mentioning that nanomaterial based immunosensors can be used as quantitative analytical tools for detection of target biomarkers in different cancers. The immunosensors is competitive with ELISA.

2.2. Enzymatic sensors

In recent years, there has been an increasing trend in the design and development of GQDs for enzymatic biosensing. GQDs and N-GQDs have been considered as an interesting material for the immobilization of desired enzymes because of some superior properties: electro-conductivity, non-toxicity, biocompatibility and ease of synthesis. Enzyme-immobilized GQDs could potentially lead to unique properties such as large surface area, high bioactivity, and excellent stability. GQDs have been widely employed in electrochemical biosensors as nanosized supports for the immobilization of analytical biomolecules. In particular, the immobilization of enzymes on the surface of these nanoparticles offers numerous advantages including enhancement of the enzymatic activity and reduction of the mass-transfer processes associated with the recognition of substrates by enzymes.

For example, Ju and co-workers suggested non-surfactant capped Au NPs can supply naked catalytic surface with highly electrocatalysis activity. The AuNPs-N-GQDs exhibit high sensitivity and selectivity for electrochemical discovery of hydrogen peroxide ($H_2O_2$) with a sensitivity of 186.22 µA/mM cm$^2$ and low detection limit of 0.12 µM. Significantly, the AuNPs-N-GQDs-based electrochemical biosensor has displayed great potential applications for discovery of $H_2O_2$ levels in human serum samples and that released from human cervical cancer cells with acceptable consequences. The current project illustrates that the Au NPs–N-GQDs nanocomposite is assurance for invention of non-enzymatic $H_2O_2$ biosensors. Such electrochemical sensor has also promising application in physiological and pathological studies [22].

As for practical applications, few enzymatic biosensors appear to be commercially feasible except for some blood glucose and hand-held immunosensors. To our best knowledge, no commercial enzymatic biosensor based on MNPs has been reported until now.
Therefore, to commercialize the enzymatic biosensors based on N-GQDs, efforts should be made to break some key technical barriers such as controlling the morphology of N-GQDs on device, realizing efficient enzyme immobilization and keeping enzyme long-life bioactivity as well as reducing matrix interference and sensor fouling. Firstly, controlling the morphologies of N-GQDs for biosensor design is necessary because the morphology of the nanosized material is one of the most important factors to determine the properties for biosensor applications. N-GQDs usually demonstrate size dependent as well as shape- and structure-dependent optical, electronic, thermal, and structural properties. Many synthetic methods have been developed for controllable preparation N-GQDs, however, for practical biosensor design, the procedure of controllable synthesis and then deposition the nanoparticles on device is not a good choice because of the tendency aggregation of nanoparticles. Thus, in-situ synthesis, for example, electrochemical deposition, CVD, template synthesis and so on, might be powerful tools to grow controlled N-GQDs on device directly. Secondly, how to realize efficient enzyme immobilization is a very critical question that should be addressed for the commercialization of biosensors. Conventional strategies, including physical or chemical immobilization, have their shortage for controlling the orientation of the enzyme in order to implement efficient enzyme immobilization and retain the long-life bioactivity of enzyme. Recently, the coupled use of site-directed mutagenesis and immobilization has been proved a very useful strategy that controlling the orientation of the enzyme and improving enzyme features from activity to stability. It should be further studied to test whether site-directed mutagenesis and immobilization enzymes in N-GQDs could improve enzyme features or not. Thirdly, for practical application of N-GQDs based enzymatic biosensors, problems associated with matrix interference and sensor fouling should be resolved. Biosensors might have well performance under controlled environments or laboratory samples, however, in real sample analysis, the matrix interference and sensor fouling are the detrimental factors for commercialization. Especially, the large surface to area for nanoparticles might lead to more serious sensor fouling. Thus, more effort should be made to solve these problems.

On the other hand, nanoparticles have emerged as versatile tools for generating excellent supports for enzyme stabilization due to their small size and large surface area. By proper surface modification, various N-GQDs have been synthesized and successfully utilized for protein/enzyme immobilization, which have already displayed promising effects in practical applications. We have summarized in this sub-section the applications of N-GQDs in enzyme immobilization, protein separation/purification, medical science and food analysis. The immobilized proteins/enzymes generally show better stability towards pH and heat than the free ones and can be recovered and reused multiple times. However, the activity of some enzymes decreases to some extent after immobilization, which indicates that more efforts are still required to explore the immobilization techniques.

2.3. Aptasensors

Aptamers are aim affinity nucleic acids chose artificially by a combinatorial series. They have regularly been suggested as alternatives to antibodies with extra wished features. In the last decades, due to a several of materials with particular features for sensor progress, aptasensors have been given more attention for developing new analytical equipment.

Interactions of nucleic acids with several nanomaterials permitted enhanced designs of biosensors. For instance, physical adsorption of aptamers on carbon-based materials resulted in numerous aptasensors, this simplifying the producing series of many optical or electrochemical sensors. In a similar way, straightforward series for firmly subsequent attachment of aptamers to nanomaterials permitted the development of sensitive and reusable aptasensors based on fluorescence, colorimetric, luminescence, quartz crystal microbalance (QCM), interferometry,
Plasmon resonance or assigned surface plasmon resonance and electrochemistry. The most popular surface functionalization consist of covalent, gold-thiol, or biotin-streptavidin attachments. Four basic techniques were used during the invention of aptasensors; (i) target-induced structure changing or (ii) displacement, (iii) sandwich, and (iv) competitive modes.

Electrochemical aptasensors (aptamer-based sensors) facilitate plain, effective and fast discovery of biomolecules which are significant in medicine, environment and food applications. At a recent day Liu and co-workers reported a sensing platform for chloramphenicol (CAP) discovery was made using N-GQDs as transducer sorts and label-free aptamer as biological recognition element. N-GQDs, combined by a facile one-step hydrothermal technique, were explored to gain highly efficient photon-to-electricity conversion under visible light irradiation. As a consequence, the PEC activity of GQDs was progressed by nitrogen doping. Additionally, the π-conjugated structure of N-GQDs supplied an outstanding platform for aptamer immobilization via π-π stacking interaction. Such an aptamer/N-GQDs based sensor displayed a linear PEC response to CAP focus in the range of 10–250 nM with a detection limit of 3.1 nM [24].

In conclusion, electrochemical aptasensors have mainly been reported for the detection of different target analytes. Detection of proteins rather than small molecules has been more developed. Weaker affinity interactions exist between aptamer and small ligands (Kd in the mM range) compared to large molecules (Kd in the range picomolar to nanomolar). Generally, detection limits for small molecule detection are in the low mM range. For instance, label-free impedometric aptasensors for the detection of small targets suffer from minor change of the interferential electron transfer resistance, so that displacement assays have been envisioned to enhance the sensitivity.

The application of aptamers in electrochemical biosensing is still in earlier stage of development. Several problems should be overcome in order to prepare aptasensor applicable for detecting analytes in complex samples. The aptamers configuration is sensitive to the salt composition; therefore liquid composition may affect the aptasensor properties. Several proteins may interact with DNA aptamers non-specifically. They could bind to the sugar-phosphate backbone of DNA and thus mask the specific binding of analyte. The presence of nucleic acid in the biological liquids may cause hybridization with aptamers and thus affect the aptamers conformation and maintaining the proper binding site. It should be also mentioned that currently only approx. aptamers are available, while the number of various antibodies is much larger. The continuous growth of immune test is also due to lack of aptamer based kits at the market. Thus, despite the advantages of aptamers over antibodies further effort is required for wide spreading aptamers based technology in practical applications. Given the rapid pace of advances in this field, the development of miniaturized, easy-to-use electrochemical aptasensor diagnostic systems for large scale clinical testing seems a realistic goal.

Also, the potential of aptasensors is immense, and this exciting area is on the brink of exponential growth. The ability to develop affinity based detection systems of tailor designed characteristics, which can be applied to analysis of analytes, unlimited, for example, by size, toxicity and matrix effects, offers the field of biosensing the opportunity to explore new and dynamic routes of sensor development and the availability of commercial aptasensors by the end of the decade can defiantly be anticipated.

2.4. Cytosensors

Considering the vital role of cells in life science and human health, cytosensors has become a hot research topic within the past years. Nowadays, electrochemical cytosensors attract much attention in the field of cytosensors. In the case of application of N-GQDs for electrochemical cytosensing, Xi et al. reported the design and invention of a new sort of electrocatalyst based on Pd nanoparticles (NPs) decorated double shell structure N-GQDs@N-doped carbon (NC) hollow nanospheres (HNSs), and examine their clinical application in electrochemical non-enzymatic H2O2 sensing system for ultra-sensitive and highly special discovery of extracellular H2O2 molecules released from living cancer cells. When implemented in the electrochemical discovery of H2O2, the resultant nanomaterial-based non-enzymatic sensor displayed good performance, consist of low detection limit down to 20 nM, linear detection vary up to 1.4 mM, high sensitivity of 0.59 mA mM−1 cm−2, suitable operating potential of 0 V, also good reproducibility, consistency, and anti-interference ability. Besides, it could be used in ultrasensitive and special discovery of a trace amount of H2O2 released from several living tumor cells in a normal state or under chemotherapy and radiotherapy. The consequent electrochemical H2O2 biosensor based hybrid HNSs materials illustrates appealing performances, consist of low discovery limit down to nanomole level, short response time within 2 s, also high sensitivity, good selectivity, good consistency, reproducibility and have been utilized in real-time tracking a trace amount of H2O2 secreted from several living cancer cells in a normal state and cured with chemotherapy and radiotherapy [25].

Due to the minority of research and development being on new N-GQDs-based electrochemical cytosensors, more electrochemical techniques should be involved in this area. With graphene, electrochemical cytosensors will be applied to pay more attention to the pre-warning, real-time detection of cancer disease. Unfortunately, one report (discussed above) can be seen for electrochemical cytosensing based on N-GQDs. Similar to many other nanomaterials used in cytosensing, the toxicity of N-GQDs is closely associated to its surface functionalization. As-prepared N-GQDs, although water soluble, is not stable in physiological solutions and causes dose-dependent toxicity both in vitro and in vivo. In marked contrast, N-GQDs with biocompatible coatings exhibits excellent stability in the presence of high-concentration salts and proteins, and appears to be less toxic in vitro and in vivo. The in vivo bio distribution of graphene is also highly dependent on its biosensing. However, how the size of N-GQDs, which is another important parameter, affects the in vivo behaviors of graphene requires further investigation. A lot more systematic explorations are demanded in order to fully understand the in vivo long-term fate and toxicology of graphene at different doses in various animal models before any clinical applications of this novel nanomaterial. In general, with the wide range of morphologies, coatings, and hybrid structures available for electrochemical cytosensing, more detailed and longer-term studies are required before serious in vivo biomedical graphene applications are implemented.

2.5. Other paper of interest

Doping carbon nanomaterials with heteroatoms can effectively tune their intrinsic features, consist of electronic characteristics, surface and local chemical properties. The N atom, having a comparable atomic size and five valence electrons for bonding with carbon atoms, has been extensively utilized for chemical doping of carbon nanomaterials. For example, N-doped carbon nanotubes (N-CNTs) displayed highly effective electrocatalytic activities for the oxygen reduction reaction (ORR) [26]. In a similar way, doping of graphene with substituent N heteroatoms could effectively modulate the band gap of graphene to introduce novel features for tool applications [27,28]. Along with several techniques developed for the preparation of N-doped graphene materials [29–30], in this
reported, used chemical vapor deposition (CVD) to invent N-doped few layer graphene sheets, which displaying great electrocatalytic activity identical to that of N-doped CNFs. In view of the striking quantum-confinement and edge impacts of 0D GQDs, doping GQDs with chemically bonded N atoms could extremely modify their electronic features and offer more active places, so making novel phenomena and unexpected features. In this reported, aware, however, no effort has been produced to combine N-doped GQDs, and for this reason, their particular optoelectronic features are nearly totally unknown. In line with the vigorous research on GQDs and N-doped carbon nanomaterials, in this reported, for the first time report here an electrochemical approach for the facile preparation of N-GQDs. Unlike their green-luminescent N-free counterparts of identical size (2–5 nm), the N-GQDs with N/C atomic ratio of ca. 4.3% emit a distinguish blue luminescence. Moreover, N-GQDs supported by 2D graphene sheets can be utilized as a fresh category of metal-free electrocatalysts for the ORR comparable to those of commercial Pt/C electrodes, N-doped CNFs, and N-doped graphene sheets.

Interesting Cai and co-workers reported, N-GQDs are combined at low temperature as a novel catalyst permitting electrochemical discovery of 2,4,6-trinitrotoluene (TNT). N-GQDs are made by an octadecyne ultra-sonication of graphene oxide (GO) forming nanometers-sized sorts, which are then chemically decreased and nitrogen doped by reacting with hydrazine. The combination N-GQDs have an average diameter of ~2.5 nm and N/C atomic ratio of up to ~6.4%. To discovery TNT, TNT is first accumulated on N-GQDs modified glassy carbon electrode by holding the electrode at a 0 V versus Ag/AgCl for 150 s in an aqueous TNT solution. Next, the N-GQDs/GC electrode with accumulated TNT is carried to a new PBS solution, where the TNT reduction flow at ~0.36 V in a linear scan voltammogram displays a linear response to TNT focus in the aqueous solution from 1 to 400 ppb, with a correlation coefficient of 0.999, a detection limit of 0.2 ppb at a signal/noise of 3 [31].

Recently Chen and colleagues reported complexation of N-GQDs with ruthenium ions probably happened between the pyridinic nitrogen dopants, leading to the incorporation of several metal centers within the combined graphitic C sp² scaffolds (Ru-NGQDs). In comparison with the pristine NGQDs, Ru-NGQDs exhibited a red-shift of the absorption band in UV–vis measurements, along with importantly decreased intensity of the photoluminescence emissions, ascribed to quenching by the metal nitrogen moieties. Electrochemically, the Ru-N-QD combines exhibited two pairs of voltammetric waves, with a peak spacing of 150 mV, proposing Class II intercalation charge transport. This was further verified in near-infrared spectroscopic measurements where an absorption band appeared at ca. 1450 nm at mixed valence by utilizing Ce(SO₄)₂ as the oxidizing reagent. The consequences emphasize the particular applications of graphene scaffolds in facilitating nanoscale charge transport [32].

Li and co-workers reported a new N-doped graphene quantum dots decorated N-doped carbon nanofibers (NGQDs@NCNFs) composite and its application to make highly sensitive electrochemical sensor for nitrite assignment. The NGQDs@NCNFs composite was prepared by mixing electrosprining, carbonization and a facile way of one-step hydrothermal technique. The as-prepared composite possessed free-standing film structure and high flexibility, which was suitable for electrode alteration. The electrocatalytic function of the NGQDs@NCNFs composite was assessed by cyclic voltammetry and electrochemical impedance spectroscopy. It is showed that the electrochemical nitrite sensor developed based on the new NGQDs@NCNFs composite exhibited great analysis function to previously stated nitrite sensors, such as extensive linear range 5–300 µM, low detection limit 3 µM, outstanding reproducibility and selectivity, and good testing recoveries for the discovery of nitrite show in sausage, pickle, lake water and tap water. These consequences propose that NQGQDs@NCNFs composite could be a promising and convenient material for the invention of electrochemical sensors [33].

Recently Shinde et al., reported a striking transformation of nitrogen-doped multi-walled carbon nanotubes (MWCNTs) to size selective N-GQDs by a two-step electrochemical method. The sizes of the N-GQDs strongly depend on the applied anodic potential, moreover increasing potential consequence in a smaller size of N-GQDs. These N-GQDs show many unusual size-dependent optoelectronic and electrocatalytic features. The presence of N dopants in the carbon framework not only causes faster unzipping of MWCNTs but also supplies more low activation energy place for improving the electrocatalytic activity for technologically daunting reactions like oxygen reduction. The smaller size of N-GQDs has displayed better function as compared to the large N-GQDs. These consequence in the size-dependent electrocatalytic activity of N-GQDs for ORR as showed by the smaller sized N-GQDs (2.5 ± 0.3 nm) definitely promising metal-free electrocatalysts for fuel cell applications [34].

3. Conclusion and perspective

N-GQDs are graphene family that have proved easy to obtain. Their exceptional optical and electronic properties and the presence of high number of reactive sites make N-GQDs powerful tools in analytical nanoscience and nanotechnology. At the same time, their opto-electronics properties make them excellent nanomaterials for a very wide variety of applications.

We have reviewed some of articles which proved that N-GQDs have great potential in the development of biosensor. Based on above discussed articles, we believe that N-GQDs based electrochemical biosensors need to be developed to detect different target analytes, elaborating microchip systems to develop GQD-based lab-on-a-chip devices for separation and detection of macromolecules, DNA, proteins and others bio-targets.

The hybridization of N-GQDs with organic or inorganic materials [e.g., polymers, CNTs, and NPs (carbon or metallic)] through covalent binding could considerably extend the possibilities for N-GQDs based bio sensor development. There is still much work to do to understand and to utilize fully N-GQDs in biosensor development and applications. Because, N-GQDs increase the contact area with the analyte, so they could increase the electrochemical active surface to interact with electroactive analytes. Since the increase in geometric surface area is very important parameter in enzymatic reactions, therefore modification of different substrates (such as glass, carbon, graphite etc.) by GQDs can increase the rate of electrochemical reaction. Therefore, integration of some modification agents into GQDs can be increases Faradic currents.

The potential incorporation of these N-GQDs in commercial biosensor increases human exposure daily. N-GQDs have been defined as low toxic, biocompatible NPs; however, their easy dispersion due to their high water solubility, N-GQDs could accumulate in the human body (organs and tissues). It is therefore necessary to develop new analytical methods that allow the determination of N-GQDs in complex matrices, such as the biological samples. There is no doubt that new strategy for surface modification need to be developed for application in biosensing. With their uniform size, excellent PL and high quantum yields, N-GQDs will no doubt be used in more creative applications. Although the advances are exciting and encouraging, the use of N-GQDs for biosensing applications is still in infancy, with a lot of challenges remaining. In summary, N-GQDs emerge as a novel nanomaterial platform for biosensing, yet many challenges need to be solved by
effective collaborations crossing multiple disciplines including chemistry, physics, biology and medicine.

Finally, we conclude that there is a bright opportunity for further advances and developments of biosensors and related devices based on N-GQDs, especially through further miniaturization and integration into lab-on-chip systems. The design of implantable biosensors with the ability to monitor cell lines in vivo and in real time is promising for the application of biosensors, even though it is yet to be explored. Scientists therefore have a great deal to do to address the performance of biosensors for cell detection in the future.

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References
