

GRAPHITIC CARBON NITRIDE QUANTUM DOTS: SYNTHESIS AND APPLICATIONS

Dang Dinh Khoi

Ho Chi Minh City University of Technology and Education, Vietnam

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ABSTRACT

Graphitic-carbon nitride quantum dots (g-CNQDs), a rising star in the carbon nitride family, has shown great potential in many fields including chemical and biomedical applications due to their good biocompatibility, stable fluorescence, high quantum yield, and nontoxicity. For this reason, enormous efforts have been devoted to optimizing synthetic methods and structures of g-CNQDs to discover the inner properties and structural features in the intriguing system. Also, a vast number of studies have been pursued to discuss the potential applications of g-CNQDs in chemical and biomedical areas. In this review, recent advances in synthesis and applications of g-CNQDs were summarized and the future challenges as well as opportunities of these g-CNQDs in the chemical and biomedical fields will be highlighted.

Keywords: *graphitic carbon nitride quantum dots (g-CNQDs); nanoparticles; fluorescence; chemical sensing; biomedical applications.*

1. INTRODUCTION

In the last decade, metal-free carbon-based quantum dots (QDs) including carbon quantum dots (CQDs) and graphene quantum dots (GQDs) have been considered to be the promising candidates to replace traditional semiconductor QDs for chemical and biomedical applications because of their excellent optical properties including good photostability, excellent biocompatibility, nontoxicity, tunable photoluminescence (PL), and also easy synthesis [1-6]. As a kind of metal-free 2D material, graphitic carbon nitride (g-C₃N₄) has been extensively studied by researchers since being a theoretical prediction by Liu et al. [7, 8]. However, because of its larger size distribution as well as poor dispersibility, the chemical, as well as biomedical applications of bulk g-C₃N₄ have been badly limited. Therefore, great efforts have been made to fabricate nanoscale g-C₃N₄ including g-C₃N₄ nanosheets and g-C₃N₄- quantum dots (g-CNQDs) [9, 10]. A kind of metal-free analog for carbon-based QDs, g-CNQDs have

shown great potential for chemical and biomedical applications [11, 12]. Similar to carbon-based QDs, g-CNQDs have advantages of small size distribution, excellent biocompatibility, good water solubility, easy functionalization, and also chemical inertness. Moreover, g-CNQDs have the conjugated tri-s-triazine structures with sp² C-N cluster, demonstrating great potential as a drug carrier for chemotherapy [13]. Differ from other carbon-based QDs, the g-CNQDs emerge as excellent fluorophores due to their unique combination of many key merits including expanded emission spectral coverage, high quantum yield, and explicit PL mechanism [14-16]. In addition, g-CNQDs have shown broad applications in chemical and biomedical areas including chemical sensing, biosensing, bioimaging, and responsive drug carrier and phototherapy by rational selection of molecule precursors or suitable preparation route and reasonable post-treatment.

In this review, we aim to provide a comprehensive on the synthesis methods of

g-CNQDs and their chemical and also biomedical applications in chemical sensing, biosensing, bioimaging, and cancer therapy. It is believed that this review will be of great interest to investigators and practitioners in materials science, biochemistry, biomedical engineering, and nanomedicine.

2. SYNTHETIC METHODS

The g-CNQDs are not just rich nitrogen-doped carbon nanomaterials comparing to carbon-based QDs. They are polymeric materials consisting of C, N, and some H impurities, which are formed through tri-s-triazine-based patterns. The unique structure of g-CNQDs has endowed them superior stability in various chemical and physical environments including acid or base media. Recently, great efforts have been made in the preparation of g-CNQDs. The strategy of synthesizing g-CNQDs is similar to that of carbon-based QDs, which can be classified into two routes depending on the size development of the precursors: top-down and bottom-up routes. For the top-down route, the synthesis of g-CNQDs is started from macroscopic g-C₃N₄ structures, followed by a series of treatments including chemical oxidation, sonication, chemical tailoring, hydrothermal treatment, electrochemical oxidation, and other approaches to form 2D nanosheets, 1D nanowires or nanoribbons, and finally to obtain 0D QDs. For the bottom-up route, g-CNQDs are obtained using carbon and nitrogen sources as a precursor by hydrothermal or solvothermal method, microwave method, microwave-assisted solvothermal method, and solid-phase method. In this section, the design and synthesis structure of the g-CNQDs synthesized using different approaches and precursors will be described.

2.1 Synthesis of g-CNQDs via “top-down” routes

The top-down fabrication of g-CNQDs includes the multi-step procedures containing the first synthesis of bulk g-C₃N₄ nano-structure as precursor template

following the miniaturization of precursor template through successively cutting of bulk material to form nanoparticles g-CNQDs [17,18]. Because the preparation of bulk g-C₃N₄ templates are performed at high temperature (>400°C), such synthetic methods often require high energy consumption. The preparation of the bulk g-C₃N₄ by using nitrogen-rich precursors is not discussed in this section for clearly focusing on the synthesis and chemical and biomedical applications of g-CNQDs [19]. Although the as-prepared g-CNQDs by the top-down route manifest complex processes, high cost, use of strong acid, base or oxidizer, and imperfection of structure, there are still some advantages intriguing, including simple operation, better uniformity, and large-scale preparation [10,20,21].

2.1.1. Chemical oxidation.

Previous publications have shown that introducing strong acid can produce the simultaneous protonation and exfoliation of the bulk g-C₃N₄, subsequently enabling g-CNQDs with hydrophilic groups [22,23]. Recently, Song et al. conducted the fabrication of g-CNQDs solution by the first chemical oxidation of bulk g-C₃N₄ using strong oxidized acid HNO₃, followed by hydrothermal treatment and ultrasonic exfoliation [24]. Due to the abundance of functional groups (hydroxyl, carboxylic acid, and amine) on their surface, the as-prepared g-CNQDs (with a diameter range from 1 nm to 5 nm) were highly dispersible in water. Taking advantage of the instability of hydrogen-bond of tri-s-triazine units in the g-C₃N₄ layers against oxidation, the chemical exfoliation is suitable for large-scale synthesis of g-CNQDs. However, this approach for the preparation of g-CNQDs is often accompanied by other treatment processes. Usually, the post-treatment of the g-CNQDs prepared by chemical exfoliation route is relatively complex due to a required step of removing the excess amount of oxidant (such as HNO₃) from the reaction media.

2.1.2. Ultrasonication

Nowadays, an ultrasonication route has been developed as a suitable method to prepare small-sized nanoparticles. Ultrasonication route of g-CNQDs was applied in a solvent that has similar surface energy to that of bulk g-C₃N₄ at ambient temperature [25,26]. The as-prepared g-CNQDs have exhibited a similar structure and stoichiometric ratio compared to bulk g-C₃N₄. To date, numerous worldwide researchers have applied the ultrasonic

exfoliation treatment of bulk g-C₃N₄, leading to the formation of g-CNQDs [27-29]. Xie et al. highlighted the synthesis of single-layered g-CNQDs that can be mainly divided into three steps, including acid treatment of bulk g-C₃N₄, exfoliation of g-C₃N₄ to form ultrathin nanosheets by hydrothermal treatment under the aid of NH₃·H₂O, and finally the ultrasonication of ultrathin g-C₃N₄ nanosheets to obtain single-layered g-CNQDs in water. The whole schematic illustration has been shown in Fig. 1. [30].

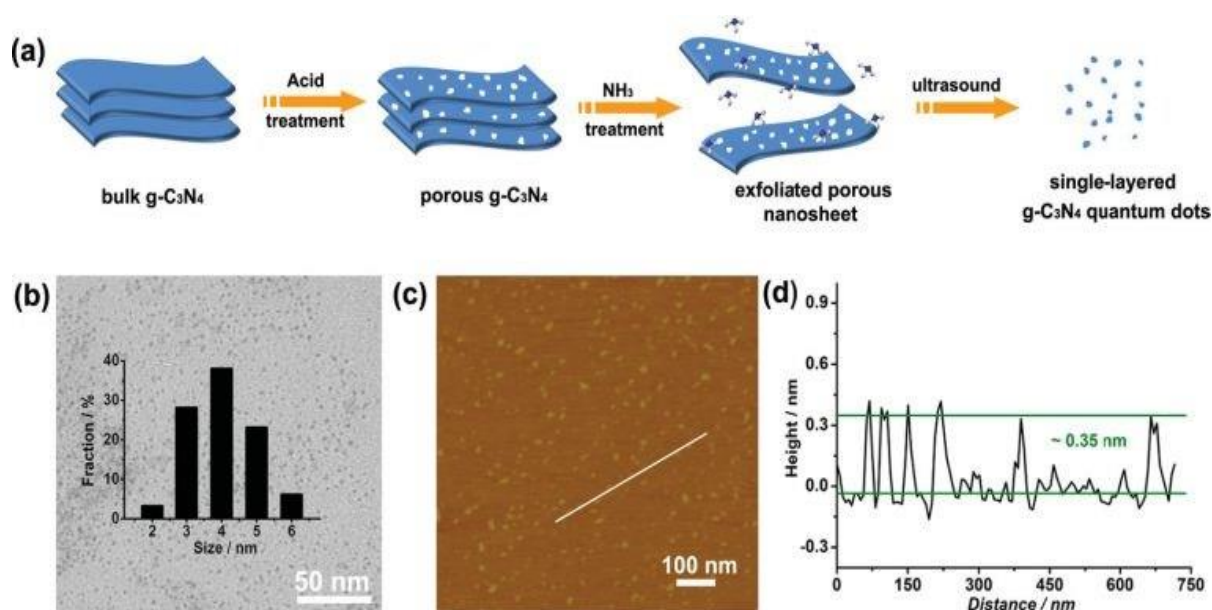


Fig. 1. (a) Schematic illustration of the strategy for the preparation of single-layered g-CNQDs. (b) TEM image and the corresponding size distribution of the g-CNQDs. (c) and (d) AFM and corresponding height image of the g-CNQDs [30].

2.1.3. Chemical tailoring.

Chemical tailoring (also preferred as chemical cleavage) usually means that a molecule has a unique effect on a certain chemical bond. The molecule can be used as precise scissors for cutting the expected molecular [31]. This route is a fragment-based method that the large molecular system under study is being cut into the expected smaller fragments. By employing a chemical tailoring process, Zhang's group has demonstrated that bulk polymeric carbon nitride could be utilized as a layered precursor for the preparation of carbon

nitride nanostructures such as nanorods, nanoleaves, and g-CNQDs [18]. Fig. 2a has demonstrated that with the help of free protons the tris-s-triazine-based compounds tend to hydrolyze into small molecules, using water as protic solvents to destroy the hydrogen bond of bulk g-C₃N₄ in a solution of H₂SO₄ and induce the partial hydrolysis of the bulk C₃N₄ (Fig. 2b). As a consequence, a C₃N₄ mixture with different nanostructures will be obtained including g-CNQDs, nanoleaves, and nanorods. After being treated by simple centrifugation and purification, the uniform g-CNQDs with a size range from 2-4 nm could be obtained.

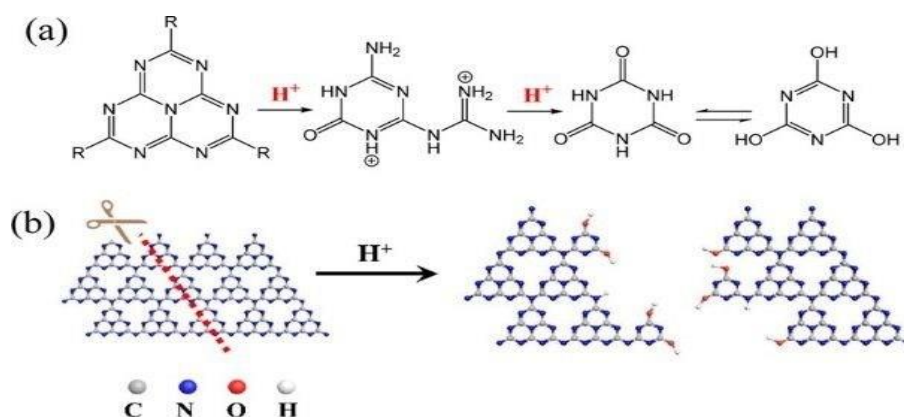


Fig. 2. (a) Proposed mechanism for the cleavage of tris-s-triazine based compound in acidic aqueous solution. (b) Scheme of chemical tailoring of graphitic phase polymeric carbon nitride [18].

2.1.4. Hydrothermal treatment

Hydrothermal cutting has been widely developed for preparing carbon-based QDs using large-size raw materials as precursors because it is a simple and green approach without the employing of organic solvents. Nowadays, some examples have been reported about the hydrothermal preparation of g-CNQDs using bulk g-C₃N₄ as a precursor. For the first time, Zhang and colleagues reported the preparation of blue g-CNQDs by hydrothermal cutting of bulk

g-C₃N₄ at 180°C for 10 h [32]. Another research conducted by Yu and co-workers has shown that g-C₃N₄ nanosheets, nanoribbons, and g-CNQDs can be controllably prepared from bulk g-C₃N₄ [33]. Fig. 3 shown that the g-C₃N₄ nanosheets from heat etching of the bulk g-C₃N₄ were degraded into g-C₃N₄ nanoribbons using the acidic cutting, and then using g-C₃N₄ nanoribbons as precursor through the hydrothermal treatment at 200°C for 10 h to obtain the g-CNQDs.

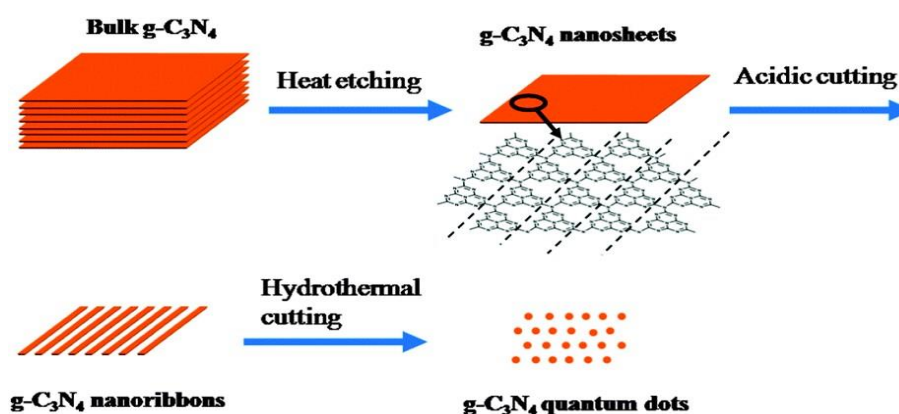


Fig. 3. Schematic illustration of the controllable synthesis of g-C₃N₄ nanosheets, nanoribbons and g-CNQDs [33].

2.1.5. Electrochemical oxidation.

Electrochemical oxidation has been proved as a useful route for introducing functional groups to the surfaces of g-CNQDs. For the first time, Zhang et al. prepared a kind of g-CNQDs with high

reducibility by using electrochemical processes from bulk g-C₃N₄ using an alkaline solution. The as-prepared g-CNQDs have a size distribution from 5 nm to 8 nm and excellent dispersion stability in water [34]. In addition, Zhang and co-workers have developed oxygen and sulfur co-doped

g-CNQDs by a simple electrochemical approach. The as-prepared g-CNQDs have a size distribution from 1 nm to 4 nm, a high quantum yield of 33.9 %, and also good water solubility which is enabled by the abundant functional groups on the surface of g-CNQDs [35].

2.2 Synthesis of g-CNQDs via “bottom-up” routes

The bottom-up route is a one-step method for the preparation of carbon-based QDs by using organic molecules as precursors. Generally, the decomposition of organic molecules contains three steps, including the organic molecules form macromolecules intermediates through a condensation, then the chemical bonds in macromolecules suffer destruction leading to the formation of carbon-based fragment or free radical, and finally the carbon-based fragment occurs the carbonization process to form nanosized carbon-based QDs. Also, free radicals can react with carbon-based QDs to produce functional groups in the surface carbon-based QDs with fewer defects. The bottom-up synthesis of g-CNQDs, which is realized by a one-pot route without additional use of bulk g-C₃N₄, should have a similar formation process via the pyrolysis or carbonization of some molecule precursors because it is a type of carbon-based QDs. To date, various kinds of nitrogen-rich organic molecules have been employed as precursors such as formamide, N, N-dimethylformamide (DMF), melamine, guanidine hydrochloride, urea, dicyandiamide, and organic amines [36, 37].

In the bottom-up route, the target atoms can be used as the building blocks for the simple creation of heterogeneous doping, which can provide precise control in the nanostructure of g-CNQDs depending on the properties to be engineered

2.2.1. Hydrothermal method

Hydrothermal treatment of organic molecules dispersed in an aqueous solution that occurred in a sealed container at high temperature and pressure [38, 39]. This kind of treatment refers to a chemical reaction using water as a dispersing agent; therefore, the used precursor for the synthesis of g-CNQDs must be polar molecules because the solvent in hydrothermal treatment is polar water [40]. For example, Lu and co-workers prepared uniform O/S-g-CNQDs using thermal treatment aqueous solution of citric acid and thiourea at 200°C for 2 h [41]. As shown in Fig. 4, the heterogeneous doping of sulfur contained in thiourea provides the direct resultant g-CNQDs, which has a quantum yield of 14.5% and an average size of 2.78 nm. The functional surface groups of fluorescent g-CNQDs enable them to serve as a potential probe for biosensing. In addition, the synthesis of g-CNQDs derived from an ionic liquid by dispersing 1-butyl-3-methyl-imidazolium-tetrafluoroborate in distilled water using a facile hydrothermal approach at 200°C for 12 h was reported by Xiao et al. [42]. The as-prepared g-CNQDs have shown a narrow size distribution of 4.15 ± 1.95 nm and are highly water-soluble and also exhibited a strong PL with a quantum yield of 8.34 %.

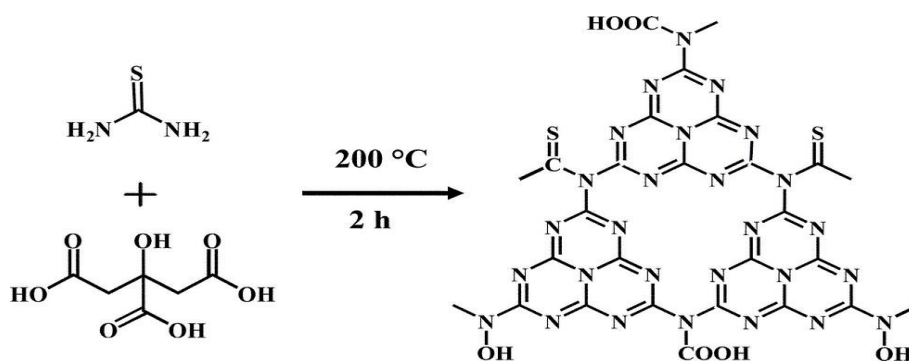


Fig. 4. Schematic illustration for the hydrothermal preparation g-CNQDs [41]

2.2.2. Solvothermal method

The solvothermal process involves the use of an organic solvent under moderate to high pressure and temperature that has a similar reaction mechanism with the hydrothermal method [43-45]. Compared to the hydrothermal method, the solvothermal treatment of organic molecules demonstrates some advantages, including the use of hydrophobic molecules for precursor, uniform size distribution, and morphology for products [46]. Moreover, the solvent in solvothermal treatment could be adjusted

according to the selection of organic precursors. For instance, Sun and co-worker reported the synthesis of g-CNQDs using 1,2-ethylenediamine as precursor and carbon tetrachloride as non-polar solvent by solvothermal treatment at 80°C for 60 min. The formation of g-CNQDs may be attributed to the polymerization of carbon tetrachloride and 1,2-ethylenediamine as shown in Fig. 5 [47]. The as-synthesized g-CNQDs have a small size distribution from 1 nm to 5 nm and pH-dependent fluorescence intensity and a quantum yield of 11 %.

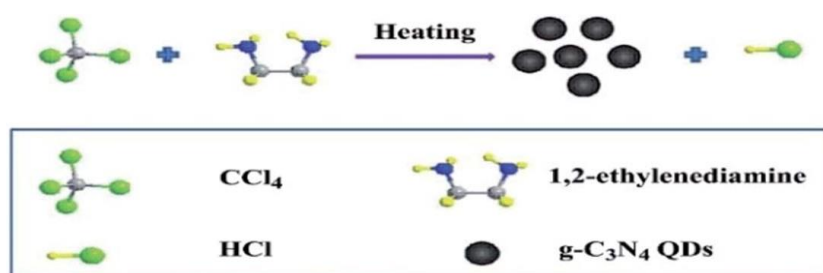


Fig. 5. Schematic illustration for the solvothermal preparation g-CNQDs [47].

2.2.3. Microwave method

In comparison with other synthetic routes, microwave-assisted synthesis of g-CNQDs not only greatly shortens sample preparation time, but also effectively reduces the occurrence of side reactions [48]. This route consumed less energy and simple operation characterized by simultaneous heating, homogeneous heating, and rapid heating, which endowed QDs with uniform size distribution and non-surface passivation [49]. The as-prepared g-CNQDs, which are uniform in size distribution and non-surface passivation, are often achieved via the polymerization of organic molecules

precursor [14, 50-53]. For example, Li et al. reported an eco-friendly and rapid microwave synthesis of green fluorescent simple and rapid one-step preparation of oxygen and sulfur dual-doped g-CNQDs using citric acid and thiourea as the precursor [54]. The as-obtained g-CNQDs showed excitation wavelength and pH-independent luminescence behaviors in the visible light. Moreover, the as-prepared g-CNQDs exhibited high fluorescence quantum yields (31.76%), strong resistance to the interference of a high ionic strength environment, and good biocompatibility as demonstrated by the cell viability assay (Fig. 6).

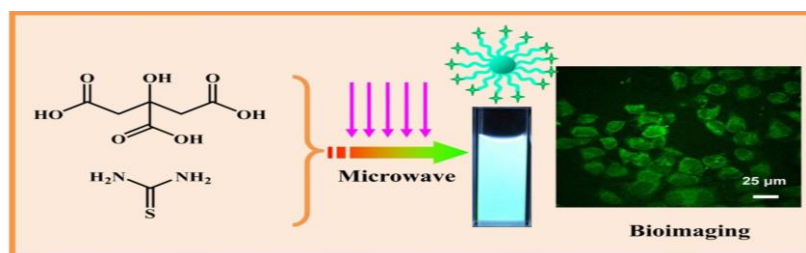


Fig. 6. Bright green fluorescent GCNQDs were facilely prepared by a simple, rapid and eco-friendly microwave synthesis method. The GCNQDs with low cytotoxicity were used for vitro bioimaging of HeLa cells [54].

2.2.4. Microwave-assisted thermal method

Microwave-assisted growth of g-CNQDs can be reduced time for reaction compared to that of the hydrothermal/solvothermal method, which is usually taking a long time, leading to fast and effective production. In addition, g-CNQDs produced using the combined method of hydrothermal/solvothermal, and microwave often possessed relatively high yield and quantum yields. For example, Wen et al. reported a process of making g-CNQDs by combining microwave and solvothermal methods in a short time (5 min) [55]. Pandey

and co-workers proposed the controllable fabrication of N-doped graphene quantum dots and g-CNQDs by adjusting the ratio of precursor citric acid to urea employing solid-phase microwave-assisted heat (SMPA) technology at 250°C for 5 min [56]. Results have shown that the atomic ratio, surface functionalization, and atomic structure of as-prepared QDs strongly depend on the ratio of citric acid to urea as shown in Fig. 7. When the ratio of citric acid to urea is 1:3, the as-synthesized g-CNQDs are obtained which have a size distribution of 3.5 nm and a quantum yield of 26.3 %.

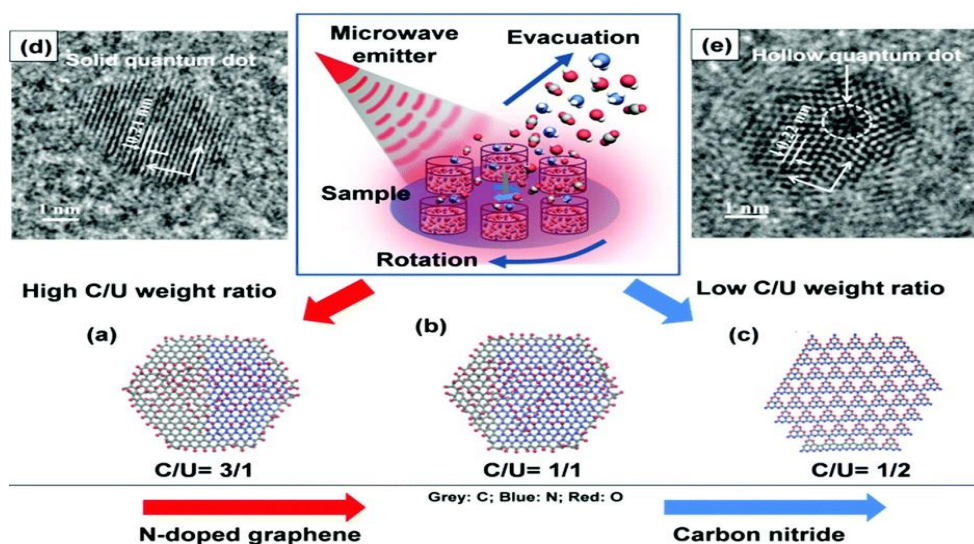


Fig. 7. Schematic diagram of two-dimensional atomic structures created during SPMA process with citric acid/urea weight ratios of (a) 3/1, (b) 1/1, and (c) 1/2, simulated by the molecular dynamics software. HR-TEM micrographs for the carbon nanodots prepared with citric acid/urea ratios of (e) 3/1 and (f) 1/2 [56].

2.2.5. Solid-phase method

In the solid-phase method, chemical reactions occur among the solid reactants, in which the old chemical bonds suffer breaking down while the new chemical bonds occur [57-60]. This process can be performed at a relatively low temperature compared to the synthesis of bulk g-C₃N₄ at high temperatures (>400°C). For example, Zhang and co-workers reported a preparation process of highly fluorescent g-CNQDs using urea and sodium citrate (with a molar ratio of 6:1) as the precursors via a low-temperature solid-phase method. The

possible mechanism for the formation of the as-prepared g-CNQDs is shown in Fig. 8 [61]. The as-synthesized g-CNQDs have a typical topographic height of 1.5-2.5 nm with a narrow size distribution of 2.6-5.5 nm and a high quantum yield of 42 %. Also, Gogoi et al. reported a type of sulfur and oxygen doped g-CNQDs using ethylenediaminetetraacetic acid disodium salt and thiourea as a precursor with a molar ratio of 1:2 through a solid-phase method at 200°C for 2 h forming as-prepared g-CNQDs exhibited a wide size distribution of 3-13 nm and a quantum yield of 13.4 % [62].

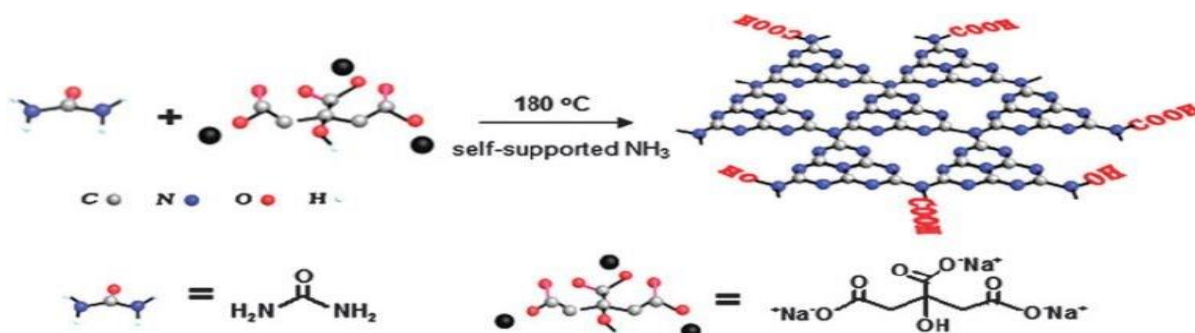


Fig. 8. Mechanism for the formation of highly fluorescent g-CNQDs using a solid-phase method at 180°C [61].

3. APPLICATIONS OF GRAPHITIC CARBON NITRIDE QUANTUM DOTS

3.1 Chemical applications

Today, the accumulation of unexpected hazardous compounds into our ecosystem is a serious problem to be solved forthwith. For addressing this problem, detection and monitoring of these compounds are crucial and fluorescent techniques provide simple and feasible detection approaches. Numerous fluorescent probes are reported in the literature based on various metal ions, organic dyes, quantum dots, semiconductors, and others. Unlike other fluorescent probes that have been fabricated so far, g-CNQDs are considered eligible candidates for fabricating fluorescent sensors because of highly stable PL against photobleaching and low toxicity.

3.1.1. Ions sensing

Having possessed several interesting properties (stability, high electron transfer characteristics, excellent PL quenching abilities, etc.), which are essential for sensing applications, g-CNQDs were employed as a promising material for developing sensors to detect various analytes. The excellent fluorescence quenching phenomenon of g-CNQDs brought new insights to utilize them as fluorescent probes for heavy metal ion detection and also small molecules. In a typical work, J. Achadu and co-workers reported a thymine-functionalized g-CNQDs

(T-g-CNQDs) via a microwave-assisted synthesis method [53]. The sensor is strongly quenched by Hg(II) and highly selective even in the presence of other metal ions due to the formation of T-Hg(II)-T base complexes (Fig. 9).

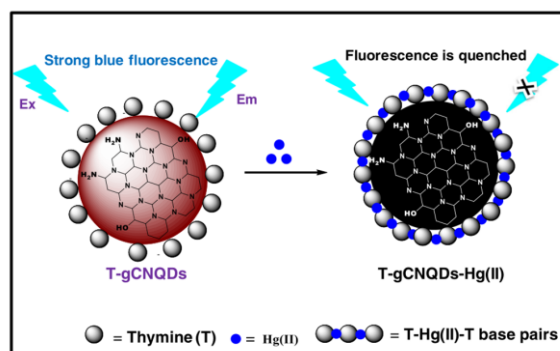


Fig. 9. Schematic representation of the detection mechanism for Hg(II) by T-gCNQDs via fluorescence quenching process [63].

Similarly, Kong et al. demonstrated a fluorometric and colorimetric dual-mode sensing nitrite (NO_2^-) in sausage and water based on a complex platform of g-CNQDs and Fe(II)-bathophenanthroline (BPS- Fe^{2+}). Because of the inner filter effect (IFE), the fluorescence of g-CNQDs was quenched by the BPS- Fe^{2+} complex. However, in the presence of NO_2^- , Fe^{2+} was oxidized by nitrite to form BPS- Fe^{3+} complex with BPS, leading to the recovery of the fluorescence from g-CNQDs. In addition, the color of the solution changed from red to colorless with the increase of NO_2^- concentration, giving measurements of the UV-vis and also on-site visual detection (Fig. 10) [63].

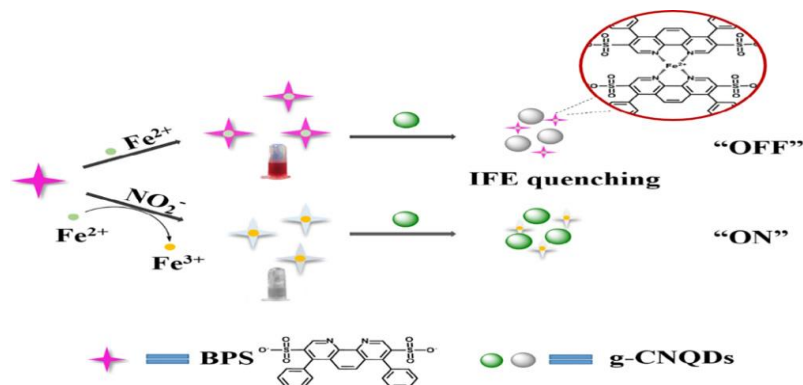


Fig. 10. Schematic illustration of the fluorescent and visual detection of NO_2^- through the IFE of BPS- Fe^{2+} complex and g-CNQDs [63].

3.1.2. Detection of organic molecules

The detection and monitoring of small organic molecules with fluorescent g-CNQDs have also become an attractive topic in recent years. The fluorescent mechanism is based on a turn-on or turn-off fluorescence response, which can be quenched directly by adding organic molecules on one hand. On the other hand, the PL probe can be quenched as an indirect mechanism which is firstly quenched by metal ions and then recovered by specific organic molecules. For instance, Lu et al. reported a facile microwave-assisted method for synthesis of functionalized g-CNQDs and applied it as a fluorescence probe for fast and highly selective detection of 2,4,6-trinitrophenol (TNT). Having fabricated by a microwave-assisted solvothermal method of L-tartaric acid and urea in oil acid media, the obtained g-CNQDs are mono-dispersed with narrow size distribution and exhibit high selectivity and sensitivity of fluorescence quenching for TNP. This sensing system exhibits a fast response time, wide linear response range, and also a low limit of detection (87 nM) which is comparable or lower than the other probes [64].

3.2 Biomedical applications

The smaller size distribution (<20 nm) and good stability of g-CNQDs endow them with rapid metabolism and excellent biocompatibility. Also, the highly π -conjugated electronic structures and

functional groups on their surface enable them as high efficient nanocarriers for targeted molecules and anti-cancer drug loading. Because of their unique structure and inner properties, the g-CNQDs have a great potential to meet the requirement of next-generation biomaterials for clinical applications. To date, various g-CNQDs have been synthesized for biomedical applications.

3.2.1. Biosensing

The properties of g-CNQDs (which are similar to that of semiconductor quantum dots) enable them as excellent optical markers for biological sensing due to the presence of the graphitic nitride-N-atom that introduces a different kind of "surface state" [53]. Various kinds of biosensors have been fabricated recently based on the tunable PL and electrochemiluminescence (ECL) of the g-CNQDs. Previously, Achadu et al. reported a microwave-assisted hydrothermal process for fabrication of tannic acid (TA)-modified g-CNQDs (TA-g-CNQDs) which have a size distribution of about 7 nm and a quantum yield of around 44 %. Interestingly, the fluorescence of TA-g-CNQDs could be quenched when the Cu^{2+} was applied and when the AA was introduced into the above solution, the fluorescence of TA-g-CNQDs could be restored as shown in Fig. 11. Such a TA-g-CNQDs-based probe allows AA to be detected with a 50 pM limit of detection and a linear analytical range that extends from 0.1 to 200 nM of AA [65].

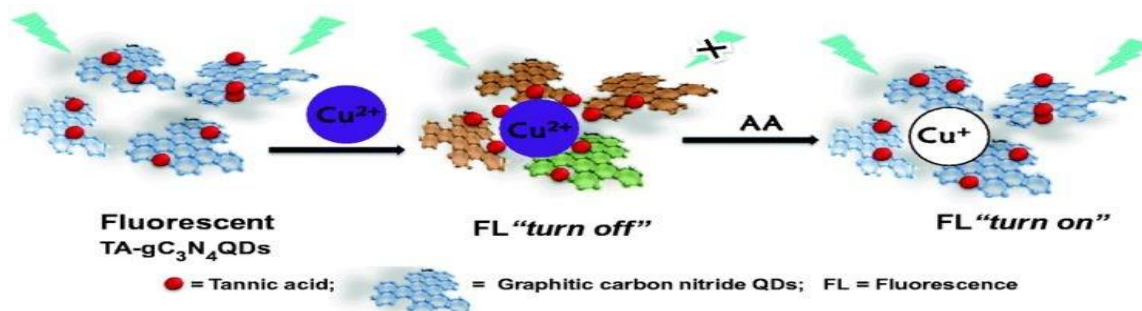


Fig. 11. Fluorescence on-off-on scheme for the detection of AA using tannic acid-functionalized g-CNQDs in the presence of Cu²⁺ [65].

Another example for biosensing is the detection of Heparin, an anticoagulant that prevents the formation of blood clots caused by certain medical conditions or procedures. Recently, Cheng and co-workers reported a g-CNQDs-based sensor for heparin detection. The sensor system is a composite composed of g-CNQDs and Ag nanoparticles which are then coated by using polyethyleneimine (PEI), leading to the

formation of Ag-g-CNQDs/PEI complexes. The coating surface has strongly reduced the PL of Ag-g-CNQDs composite at an emission wavelength of 600 nm (Fig. 12). In contrast, when heparin was added, the Ag-g-CNQDs system exhibited the obvious PL enhancement. This Ag-g-CNQDs sensor showed potential as a fluorometric assay for heparin with a detection limit of 8.2 nM [66].

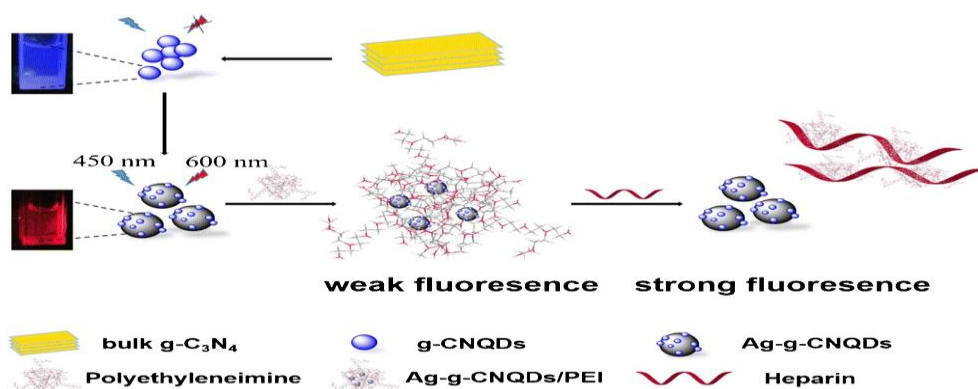


Fig. 12. Schematic illustration for fabricating metal silver nanoparticle-enhanced red fluorescence of g-CNQDs for heparin detection [66].

3.2.2. Bioimaging

Confocal imaging has many advantages, including the ability to control the depth of field, reduce background information, and the ability to collect continuous optical slices from thick specimens in comparison with conventional fluorescent optical imaging. Having advantages of excitation wavelength-tunable fluorescence emission and excellent photostability, the g-CNQDs exhibited great potential for long-term bioimaging using laser scanning confocal microscopy [67, 68].

In previous work, Zhan and co-workers reported an ethanol-thermal treatment of bulk g-C₃N₄ in the presence of KOH for producing blue-emitting g-CNQDs [69]. As shown in Fig. 13, Hela cells exhibited bright blue fluorescence at the excitation wavelength of 405 nm after being treated with g-CNQDs for 5 h. Also, the g-CNQDs exhibited good photostability and were suitable for long-term cellular imaging because no significant fluorescence quenching of Hela cells treated by g-CNQDs.

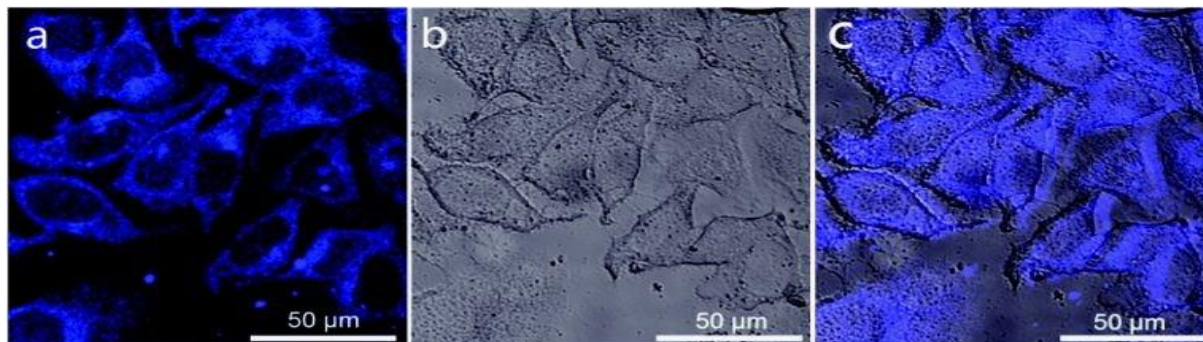


Fig. 13. (a) Confocal fluorescence microphotograph of the HeLa cells incubated with g-CNQDs for 5 h ($\lambda_{ex} = 405$ nm). (b) Bright-field microphotographs of cells. (c) An overlay image of (a and b) [69]

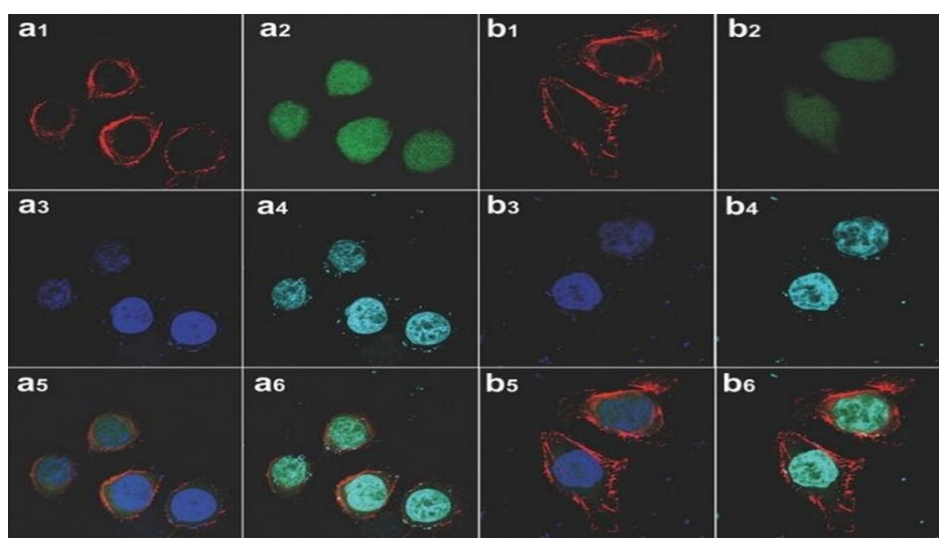


Fig. 14. One-photon fluorescence images of HepG2 cells co-stained with 1,1-dioctadecyl-3,3,30,3-tetra methylindocarbocyanine perchlorate (Dil) (a1 and b1), fluorescein diacetate (FDA) (a2 and b2), g-CNQDs (a3), and DAPI (b3). Two-photon fluorescence images of HepG2 cells with g-CNQDs (a4) and DAPI (b4). Merged images of a1–a3 (a5), a1, a2 and a4 (a6), b1–b3 (b5), b1, b2 and b4 (b6) [30].

To date, the two-photon fluorescence (TPF) has been reported by fewer researchers. As shown in Fig. 14, a promising, safe, and economic fluorescent probe based on single-layered g-CNQDs for TPF imaging of cellular nuclei was developed by Xie's group for the first time [30]. The as-prepared g-CNQDs with a uniform size distribution of around 4 nm could emit stable and strong TPF with two-photon absorption. Due to their narrow size distribution and specific interaction with chromatin, the g-CNQDs have shown great potential as a promising and economic probe for TPF imaging of the cellular nucleus.

3.2.3. Cancer therapy

Cancer treatment is the method that uses surgery, radiation, medications, and other therapies to cure, shrink or stop the progression of cancer. The application of g-CNQDs in cancer therapy including responsive drug release for chemotherapy or external light irradiation for phototherapy will be discussed thereafter.

An interesting example in cancer therapy is external light irradiation for phototherapy. Among them, NIR irradiation has gained more attention for biomedical applications due to its low energy

absorption, maximum penetration, as well as a minimum side effect [70]. Wang and co-workers reported NIR-light-triggered diagnostic and therapeutic systems of g-CNQDs [71]. As shown in Fig. 15, the defective g-CNQDs could be applied as dual-functional nanoplatforams for synchronously TPI imaging and two-photon excited PDT (TPE-PDT) under a NIR laser

with a wavelength of 800 nm. Also, Chu et al. reported a microwave-induced PDT for synthesizing g-CNQDs, which demonstrated high therapeutic efficacy because of the effective generation of singlet oxygen species [72]. In contrast, the same g-CNQDs have no cytotoxicity to the UMR-106 cells without application of microwave even at much higher doses.

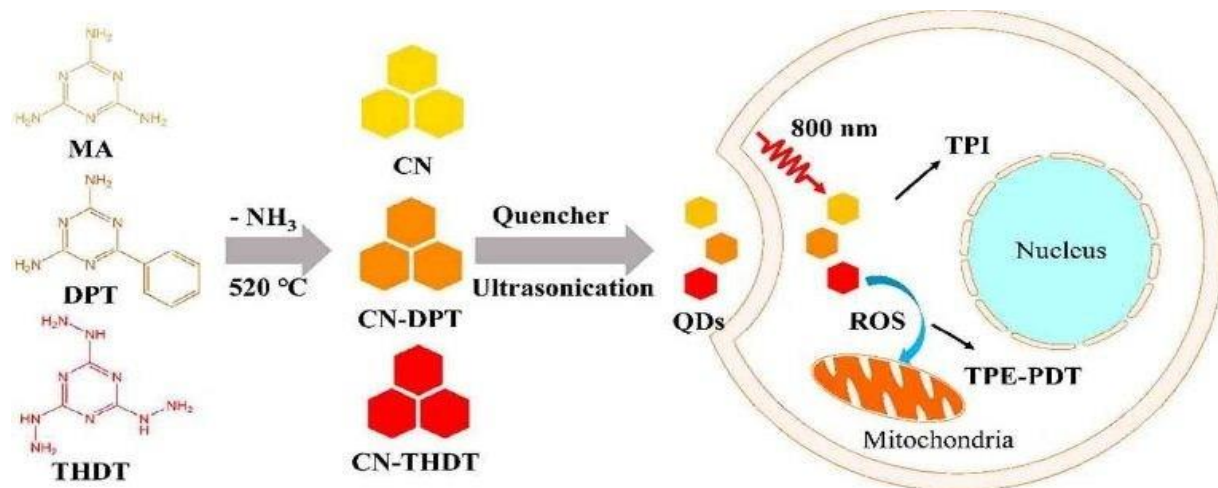


Fig. 15. Illustration of the preparation of three g-C₃N₄ QDs as dual-functional nanoplatforams for TPI and TPE-PDT in breast cancer [72].

4. CONCLUSION AND PERSPECTIVES

In conclusion, the preparation methods and chemical and biomedical applications of g-CNQDs have been discussed in this work. Emerging as novel nanoplatforams, g-CNQDs are effectively not only probed for chemical sensing of ions and organic molecules but also promised for biosensing, bioimaging, and cancer therapy as well.

However, there are still several challenges to overcome before the chemical and clinical applications of g-CNQDs. Firstly, most of the current reported g-CNQDs show blue or green fluorescent color emission while cells and biological tissues mainly contain carbohydrates and emit visible PL, which may interfere with the fluorescence signals from g-CNQDs. Therefore, long excitation and emission PL wavelengths, especially in NIR second

AND

window (>1000nm) are particularly needed for biomedical applications.

Another problem is the lack of imaging-guided combined therapy when g-CNQDs are used as therapeutic agents. Advanced routes for synthesis, suitable raw materials, inner defect structure, surface passivation, etc. can probably contribute to the improvement of the multifunction of g-CNQDs. Despite the great progress that has been obtained for cancer therapy, patients still suffer from severe side effects while treating by different methods. The integration of medical therapy with bio-sensing/bio-imaging is potentially provided for diagnosis, therapy, and monitoring of the response to treatment simultaneously for reducing side effects. It is hoped that the highlight of the latest development of g-CNQDs from its extensive synthesis methods to its wide applications in chemical and biomedical fields will help expand this infant field of research.

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Corresponding author:

Dang Dinh Khoi

Ho Chi Minh City University of Technology and Education

E-mail: khoidd@hcmute.edu.vn