Nanostructured graphene
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Chapter 7

Graphene quantum dots-synthesis, optical properties and cell imaging*

Graphene quantum dots (GQDs) exhibit unique physical and chemical properties due to their quantum confinements, edge effects and defect contents. Synthesis of GQDs with controlled defect content is an important issue for various applications. In this paper, an eco-friendly, fast and industrial promising method for synthesizing GQDs in large scale is reported via an ultrasonic-assisted liquid-phase exfoliation technique. The production yield of GQDs in N-methyl-2-pyrrolidone (NMP) can reach 3.8 mg mL⁻¹. GQDs with different sizes, structures and defects were obtained by using different graphitic carbon precursors for exfoliation. Hereby we synthesized high-defects GQDs (HD-GQDs) and low-defects GQDs (LD-GQDs) from acetylene black and nano-graphite, respectively. By luminescent and absorbance investigations, different light absorption and photoluminescence (PL) properties were identified relevant to the defect characters. The different edge structures, defect contents and sizes of GQDs are responsible for the variation of luminescent properties induced by changing the excitation wavelength and the pH values of the GQDs dispersions. Attributed to the high water dispersancy, excellent biocompatibility and controllable fluorescent performances, the as-synthesized HD-GQDs show high potential as fluorescence nanoprobes for bioimaging.

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7.1 Introduction

Graphene has been one of the most promising materials due to its outstanding mechanical and electronic properties. When cutting zero bandgap graphene into nanoribbon, it presents incredible edge effects and quantum confinement that make graphene semiconductors possible. These physical properties are also applied to graphene quantum dots (GQDs) and even become stronger as their size is reduced down to several nanometers. Apart from that, GQDs exhibit other remarkable chemical and physical properties that can be applied in optical, biological, energy, electrochemistry, ion sensing, catalysis, light emitting applications and photovoltaic devices. For example, exceptional optical properties of GQDs like photoluminescence (PL), chemiluminescence and electrochemiluminescence (ECL) have been reported. GQDs also have good biocompatibility, chemical stability and photostability under long-time laser irradiation, which make them a promising material to replace the toxic semiconducting quantum dots used for bioimaging.

Owing to their unprecedented properties and incessant new findings of GQDs, various synthesis methods of GQDs have been developed, such as nanolithography, solvothermal/microwave-assisted oxidation cutting, pyrolysis, electrochemical or mechanical-assisted chemical exfoliation, nanotomy assisted exfoliation, organic synthesis, decomposition of fullerene, etching mask on graphene and so forth. However, there are still many limitations, specifically such as complicated synthesis procedures, low production yields, expensive equipment, extreme conditions (strong acid such as H₂SO₄ and HNO₃, corrosive agents as well as strong oxidants) and high cost, which hinder their industrial application progress. Additionally, the strong chemicals may damage the original structures of graphitic precursors and introduce many other defects and groups. Thus, it is of significance to develop a facile, green, economic and efficient strategy for large-scale synthesis of GQDs.

In addition, atomic-scale defects and edge structures can significantly alter the physical and chemical properties of graphene and GQDs. For example, highly defective structures with numerous sp³ carbon and basal-plane vacancies induce poor optical properties. Thereby, controllable creation and selection of different types and densities of defects in graphene and GQDs, especially for low defects graphene derivatives, become increasingly meaningful in various applications. Polymorphic atomic defects in graphene layers were induced by energetic particles such as electrons and ions. Micromechanical cleavage of bulk graphite, epitaxial CVD growth and solvent exfoliation offered possibilities to obtain low defects and defects free graphene. Mohanty et al. reported a technique to control the shape and size of graphene nanostructures by diamond-edge-induced nanotomy and subsequent exfoliation. For most methods of synthesizing GQDs from graphene oxides (GO) as raw materials, preparing GQDs with controlled
defects content is still difficult because GO itself contains lots of defects which are randomly generated in the oxidation and exfoliation processes. Also, the cutting processes introduce lots of defects on edges. Liquid-phase exfoliation methods of graphite, such as ultrasonic assisted exfoliation, surfactant assisted, shear exfoliation, aqueous phase exfoliation, increasingly tend to be dominant for synthesis of graphene because of the low cost, industrially scalable production and good graphene quality. Coleman et al. reported that the surface energies of some organic solvents could overcome the van der Waals forces of graphite layers and thereby graphene was exfoliated from bulk graphite. The weak-energy solvent exfoliation technique also brought few damages to graphene sheets. Defects-free graphene with micrometer sizes was successfully and extensively produced by this technique.

In this paper, we further extended the solvent exfoliation method to produce graphene quantum dots. The defects content in GQDs could be simply chosen by selecting the precursor substances. This exfoliation method shows a high potential for the mass production of GQDs in low cost, fast processing and environmentally friendly way. To demonstrate the defects-density of GQDs selectable, GQDs with different sizes, edge structures and defects distribution are produced by using low-defects nano-graphite and high-defects acetylene black as the precursors, respectively. The production yield of GQDs with high content of defects (HD-GQDs) extracted from acetylene black can reach 3.8 mg mL⁻¹. Optical characterization shows that as-prepared GQDs exhibit blue photoluminescence. The pH value of different types of GQDs dispersions and excitation light dependent photoluminescence are scrutinized. For further application, the photoluminescence properties of GQDs are demonstrated as a promising fluorescent probe in cell imaging.

7.2 Experimental section

7.2.1 Chemicals

Nano graphite (NG) (40 nm in average size), N-methyl-2-pyrrolidone (NMP), N, N-dimethylformamide (DMF) were purchased from Aladdin Reagents (Shanghai) Co. Ltd. Acetylene black (AB) was purchased from Alfa Chemicals Co. Ltd. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, pH 7.4), RPMI-1,640 medium, trypsin and fetal bovine serum (FBS) were obtained from Gibco. All of the chemicals were used without further purification.

7.2.2 GQDs preparation

In a typical preparation of HD-GQDs, 300 mg acetylene black was dispersed in 30 mL NMP solvent. The dispersion was then kept under mild ultrasonication (45 W, 59 kHz) for 1 h. A grey liquid containing dispersed GQDs and some residual
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precipitates was obtained. The precipitates were removed by centrifugation at 10,000 rpm for 30 min, obtaining a homogeneous grey dispersion of GQDs. NMP could be removed by vacuum evaporation at temperature of 100 °C. The production yield was determined in terms of the dispersed concentration of GQDs in NMP, which is the ratio of dried product to the volume of NMP. Then the GQDs were dispersed in DI water immediately forming a brown solution that could be stable for months without precipitation. The same procedure was used for producing GQDs with low-content defects (LD-GQDs) except replacing AB with NG.

7.2.3 Characterization

X-ray diffraction (XRD) patterns were collected with a Rigaku D/max-2550V diffractometer (Cu Kα radiation, λ=0.15406 nm). High-resolution transmission electron microscopy (HR-TEM, JEM-2,010F) was used to characterize the structure of as-prepared GQDs. The AFM images for measuring the thickness of GQDs were obtained on a Veeco Dimension 3,100 AFM. Raman spectroscopy was conducted on a DXR Raman Microscope with a 532 nm excitation wavelength. Fourier transform infrared (FT-IR) spectra were collected with an IRAffinity-1 FTIR spectrophotometer using the KBr pellet method. X-Ray photoelectron spectroscopy (XPS) were performed through an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA) equipped with an Al source (10 kV, 22 mA) for determining the composition and chemical bonding configurations. The scans were obtained with a spot size of 250×1,000 μm. Surveys of the overall spectrum in the binding energy range of 1 to 1,200 eV were done at a low resolution (pass energy, 150 eV), after that the peaks over a 20 eV binding energy range were recorded at high resolution (pass energy, 50 eV) for C 1s, O 1s and N 1s. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. The optical data were measured on a FluoroMax@4 fluorescence spectrometer. The pH of GQDs suspension was tuned by using 10% HCl and 10% NaOH solutions.

7.2.4 Cell culture

The mouse fibroblast cell line L929 were cultured in RPMI-1,640 medium (pH 7.4) supplemented with 2.0 g L⁻¹ sodium bicarbonate, 4.5 g L⁻¹ glucose, 0.11 g L⁻¹ sodium pyruvate, 2.383 g L⁻¹ HEPES and 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin–streptomycin solution at 37 °C in a humidified incubator under 5.0% CO₂. All experiments were conducted with cells in the logarithmic growth phase. All the above procedures were carried out in a biological safety cabinet.

7.2.5 Cell viability assay

Firstly, 150 μL of the cell suspension with a density of 6.0×10⁴ cells mL⁻¹ was seeded in 96-well plates and growing overnight. Then the cells were incubated with various concentrations of GQDs samples for 24 h. After that, the culture medium was removed and washed twice with PBS for the following MTT assay. Briefly, 20
μL of 5 mg mL⁻¹ 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added into each well and incubated for 4 h. Thereafter the MTT solution was removed and the precipitated violet crystals were dissolved in 200 μL of DMSO. Finally, the absorbance was carried out at 490 nm through a Bio-Tek microplate reader.

7.2.6 Cell imaging

The fibroblast L929 was seeded in culture dishes (40 mm in diameter) and cultured in RPMI-1,640 culture medium with 2 mL dish⁻¹. All cells were incubated for at least 24 h until approximately 80% confluence was reached. Added different amounts of the aqueous solution of GQDs into culture medium and formed GQDs-contained culture medium solution with various concentrations. Following that was introducing the GQDs-contained culture medium into the cells and incubating for 2 h. After that, the cells were added with 1mL D-Hanks solution after washing triple times with D-Hanks. Finally, the culture dishes were transferred to Olympus IX71 inverted microscope for cell imaging analysis under 488 nm excitation.

7.3 Results and discussion

7.3.1 Synthesis and microstructures of GQDs with various defects

Previous work reported that graphite and carbon nanotube (CNT) could be well dispersed in some solvents, of which the surface energy matches that of graphitic surfaces. Defects-free graphene could be subsequently synthesized by the liquid-phase exfoliation and shearing methods. These results proved that graphite layers can be peeled apart by the surface energy that overcomes the van der Waals forces. The enthalpy of mixing graphitic carbon in NMP dispersion is close to zero, indicating the stable properties of NMP dispersion of graphitic carbon, which is also the reason why NMP was used in our experiments. Theoretically if the van der Waals forces between pristine graphite layers can be overcome, other less graphitized carbon materials must be easier to be exfoliated, for example small graphite particles, thermal expansion graphite and some amorphous carbon materials containing lots of graphite nanocrystallites. For this reason, the large-scale exfoliation and separation of graphene can be extended to smaller sizes, such as graphene quantum dots synthesized in this work.

The scheme shown in Figure 7.1 illustrates the process of ultrasonic-assisted solvent exfoliation used in this work. The synthesis contains three steps: (1) the first step was the wetting and intercalation of graphite particles by NMP molecular or other organic solvents under ultrasonication. Considering longer time exfoliation would cause many extra defects, we used short time ultrasonication. During this step, organic molecular attached on the surface and edges of raw carbon materials, as well as the intercalation of organic molecular into layers of raw carbon precursors to form the wetted and intercalated intermediates. As carbon black has low graphitization, the intercalation process is much easier. (2)
second step was the GQDs exfoliated from the surface unstable pieces of the intermediates. In this process, the surface energy overcomes the van der Waals forces of graphite layers, and drives the exfoliated unstable layers into NMP. Turbulence generated by ultrasonication also enhances the exfoliation process. After removing the surface unstable layers, new surface unstable layers could be generated again. In this case, more GQDs were the continuously exfoliated. (3) After exfoliation, the following step is the separation by centrifugation. In this step, the residuals including raw carbon particles, large-size intermediates, multilayer nanographene were removed and left GQDs dispersion in organic solvents. During all of the exfoliation process, ultrasonication is utilized to accelerate the exfoliating and dispersing in the organic mixture.

Figure 7.1 Schematic illustration of the preparative process of GQDs by ultrasonic-assisted exfoliating graphitic carbon precursors (acetylene black/nano-graphite) in organic solvents.

The exfoliation is proved by our experimental results of exfoliating acetylene black and nanographite to obtain GQDs as shown in Appendix Figure A5.1. After 1h ultrasonication and centrifugation, the solvents became grey or dark. In the suspension, large amounts of GQDs were produced. Different from the exfoliation process of graphene, higher speed centrifuge (e.g. 10,000 rpm) was needed to separate the GQDs from large graphene patches or residual carbon particles. The GQDs in NMP suspension could be used for two years without formation of aggregates. The lower transparence of the dispersions was related to the higher concentrations of GQDs, indicating the high degree of exfoliation or the exfoliation efficiency of the precursors. The production yield of HD-GQDs could reach 3.8 mg mL⁻¹, which is much higher than the most reported solution methods. As AB contained plenty of irregularly oriented graphite nanocrystals with paracrystalline structures, it needs weak external forces to extract GQDs but with rich defects. Different to AB, NG with higher graphitization that means stronger
van der Waals forces between the atomic layers thereby needed higher external forces to peel the layers apart. The production yield of LD-GQDs using NG was 3.1 mg mL⁻¹. Thus, different graphitization levels of raw carbon materials affected the exfoliation yields. In addition, other solvents like DMSO and DMF were also investigated. Hardly any exfoliation was obtained as no visible transparency change was observed in DMSO dispersion during ultrasonication (see Appendix Figure A5.2), while a brown solution was obtained with exfoliation in DMF. In comparison, NMP was the best solvent among them. It should be noted that ultrasonication can accelerate the exfoliation. We found no clear color change without ultrasonication after keeping the carbon precursor in solvents even for one week. In contrast, dark dispersions could be fast formed within several minutes under ultrasonication followed by centrifugation. To inhibit much damaging in the exfoliation process, mild ultrasonication (low frequency and short time) was applied.⁴⁸,⁵² When excited by ultraviolet light, the GQDs emitted bright blue light (see Appendix Figure A5.3). The high yields, abundant and cheap raw materials, fast and green processing conditions make this ultrasonic-assisted liquid-phase exfoliation method suitable for mass production of GQDs.

![Graph showing XRD patterns and Raman spectra of HD-GQDs and LD-GQDs](image)

**Figure 7.2** (a) XRD patterns of HD-GQDs and LD-GQDs. Blue curve is for HD-GQDs and black curve is for LD-GQDs; (b) Raman spectrum of HD-GQDs and LD-GQDs, respectively

Figure 7.2a shows the XRD patterns of as-synthesized LD-GQDs and HD-GQDs, respectively. The broad hump XRD peak of LD-GQDs and HD-GQDs both appear round 2-theta 22°, similar to the previous observations, indicating the disordered stacking of GQDs with sp²-carbon structures.⁸ For raw carbon materials NG and AB (see Appendix Figure A5.4), NG shows sharp and clear diffraction peaks because of its great crystallization and high graphitization, whereas AB has two broad and hump diffraction peaks indexed to (002) and (200) lattice planes because of the defect-rich nanocrystalline structure.⁵²,⁵³

Raman spectroscopy shown in Figure 7.2b confirms the quality of the as-prepared HD-GQDs and LD-GQDs. HD-GQDs show a disordered (D) band at 1,343 cm⁻¹ and a graphitic (G) band at around 1,590 cm⁻¹. LD-GQDs show a similar D band but a blue shifted G band at 1,565 cm⁻¹, which is due to the increased layers.
With respect to LD-GQDs that show a relatively sharp 2D peak at 2,690 cm\(^{-1}\), HD-GQDs exhibit a weak 2D bump, which is because the high disorder degree of HD-GQDs reduces its intensity.\(^{54}\) In addition, the disorder makes the D band increased and the G band decreased in the case of HD-GQDs. The I\(_D\)/I\(_G\) ratio was used as an evaluation of GQDs edge quality such as the relative edge roughness/defect-density.\(^{39}\) HD-GQDs gives a high I\(_D\)/I\(_G\) value of 0.966 indicating high edge roughness/defects, which are related to the precursor acetylene black that has high defects and low crystallinity. The small I\(_D\)/I\(_G\) ratio of 0.413 measured for LD-GQDs indicates the lower edge roughness/defects and good crystallinity. As far as being reported, the I\(_D\)/I\(_G\) of as-synthesized LD-GQDs is lower than most GQDs synthesized by cutting and organic synthesis methods.\(^{8,9,24,26,28,30-35}\)

**Figure 7.3a** shows Fourier transform infrared (FT-IR) spectroscopy of various carbon materials NG, AB, HD-GQDs and LD-GQDs respectively, giving the surface structure of GQDs. Compared with AB and NG, GQDs exhibit the new absorption peaks at 3,400 cm\(^{-1}\) and 1,040 cm\(^{-1}\), which are attributed to the stretching of OH of adsorbed water molecules and surface O–H groups and the C–O group conjugated with condensed aromatic carbons. Other peaks like the one at 1,288 cm\(^{-1}\) are due to the vibration of C–OH bonds, and stretching vibrations of –CH\(_2\)– lead to the peaks at 2,920 cm\(^{-1}\) and 2,850 cm\(^{-1}\). The observed peak at 1,675 cm\(^{-1}\) corresponds well to the characteristic stretching vibration of amide-carbonyl (–CO–NR) that mostly indicates the residuals of NMP in the products. The FT-IR spectra reveal that the obtained GQDs have some hydroxide and amide-carbonyl groups and may be originated from the residuals.\(^{47,48}\)

X-ray photoelectron spectroscopy (XPS) was performed to determine the composition of LD-GQDs and HD-GQDs. Figure 7.3 shows the survey spectrum of the LD-GQDs and HD-GQDs. Three peaks are observed at 532, 400, and 285 eV in the spectrum of both HD-GQDs and LD-GQDs, corresponding to the characteristic O 1s, N 1s and C 1s signals, respectively.\(^{55}\)
Figure 7.4 High-resolution XPS spectra of LD-GQDs and HD-GQDs: (a, c) C1s and N1s spectra of LD-GQDs; (b, d) C1s and N1s spectra of HD-GQDs. In each figure, the black curve is the measured spectrum, the red curve is the fitted one, and other colored curves are deconvoluted components.

The high-resolution XPS spectra of C1s in Figure 7.4a and Figure 7.4b can be deconvoluted into three components corresponding to C−C at 284.8 eV, C−(O,N) at 286.2 eV and C=O / (C=O)−NH−C at 288.0 eV.55 This indicates the surface of both HD-GQDs and LD-GQDs are functionalized with hydroxyl, carbonyl and amide-carbonyl groups. Figures 7.4c and 7.4d give the high-resolution XPS spectra of N1s, which are deconvoluted into two peaks centered at 399.8 eV and 402.4 eV, corresponding to (C=O)−NH−C and N−C3 respectively.55 The above results may indicate that the edges and surfaces of GQDs are attached with NMP residual molecular.47,48 The existence of (C=O)−NH−C group may be generated by the decomposition of NMP molecular under drying process. TGA results show that the two samples have main weight losses at 300-400 °C (see Figure A5.8 in Appendix), which is ascribed to the in-plane and edge oxygenated functionalities. According to TGA and XPS results, it is considered that the edges and other defects of GQDs could bond with the (C=O)−NH−C groups or NMP molecular. Due to these functional groups, the GQDs have good stability as dispersion in water. Table A5.1 gives the content of C, N, and O atoms in LD-GQDs and HD-GQDs as determined by XPS measurements. The total carbon consists of C−C, C−(O,N) and C=O / (C=O)−NH−C bonding while the total N consists of (C=O)−NH−C and N−C3.
carbon content of LD-GQDs is higher than that of HD-GQDs whereas the oxygen and nitrogen contents are lower than those of HD-GQDs. This is also consistent with the EDS results (see Figure A5.5, A5.6, A5.7 in Appendix and Table A5.2). The above results further prove the low defects content and better edge and surface quality of LD-GQDs with respect to HD-GQDs.

Figure 7.5 (a, c, d) HR-TEM images of HD-GQDs, with the insert of (a) being SAED pattern; (b) size distribution of HD-GQDs. The white arrows in (c) mark the zigzag edges and the lattice spacing. The red arrows of (d) mark the defects of HD-GQDs.

To further investigate the structure and defects of synthesized GQDs, high-resolution transmission electron microscopy was carried out to reveal the differences between HD-GQDs and LD-GQDs. Figure 7.5a is the HR-TEM overview micrograph of as-synthesized HD-GQDs. The inset of Figure 7.5a shows the selected area electron diffraction (SAED) pattern composed of diffuse rings, indicating the disordered character and low-crystallinity of HD-GQDs. Figure 7.5b
shows the size distribution of HD-GQDs, which is between 2 and 6 nm and most of them are 3 nm in size. The atomic force microscopy (AFM) characterization confirms the thickness distribution of 0.4-2 nm (see Figure A5.9a in the Appendix), indicating monolayer to few-layers of GQDs. In Figure 7.5c, the HD-GQDs demonstrate high edge roughness and numerous defects, which are in accordance with the Raman spectra in Figure 7.2b. The reason for HD-GQDs having much curved shape and zigzag edges is that the acetylene black is mostly spherical in shape. Figure 7.5d shows the lattice fringe of 2.4 Å space corresponding to the (11 \( \bar{2} \) 0) of graphene. In addition, various in-plane defects including single vacancy defects and double vacancy defects can be observed (marked with red arrows).
In comparison, LD-GQDs show different sizes, edges and profiles from HRTEM images, as shown in Figure 7.6. LD-GQDs have larger size and low density of defects. The bright points in the SAED pattern (inset of Figure 7.6a) confirm the good crystallinity of LD-GQDs. Figure 7.6b and Figure A5.9b in the Appendix clearly show the broader size distribution of as-synthesized LD-GQDs between 2 and 9 nm and the thickness distribution of 0.3–3 nm. In geometrical profiles, LD-GQDs show more rectangle and hexagon shapes, and thus more linear edges rather than rough curved edges that HD-GQDs present, which in turn verify the good edge quality as indicated by Raman spectra. As shown in Figure 8.6c, armchair edges are apparently observed adjacent to the zigzag edges. The lattice fringe of LD-GQDs is also 2.4 Å, corresponding to the (11 2 0) of graphene. However, no obvious in-plane defects are found for LD-GQDs samples. These results demonstrate that almost all GQDs contain mixed armchair and zigzag edges in both LD-GQDs and HD-GQDs.9,42

7.3.2 Optical properties of GQDs with various defects

GQDs open the band gap and exhibit some unique optical properties in comparison with graphene sheet of large sizes, particular in PL and ECL performances. The optical properties of HD-GQDs and LD-GQDs are investigated. Figure 7.7a shows the absorption spectra of HD-GQDs and LD-GQDs dispersed in water. The as-synthesized HD-GQDs exhibit good aqueous dispersancy and appear in weak yellow-grey color under natural light, but a blue emission when irradiated by a 365 nm lamp (Figure 7.7a inset). From the UV/Vis spectra in Figure 7.7a, there are three obvious absorption parts in the spectra at 235 nm, 283 nm, and 345 nm wavelength, respectively, for both HD-GQDs and LD-GQDs. While typical graphene only has a single $\pi-\pi^*$ transition of the C=C bond at 270 nm, thus the absorption peaks observed at around 5.28 eV ($\sim$235 nm) is attributed to the blue shifted $\pi-\pi^*$ transition of GQDs of the aromatic $sp^2$ domains. The absorption around 283 nm corresponds to the blue shifted $n-\pi^*$ transition of the C=O bond from 290–300 nm. The third transition around 345 nm is due to the trapping of excited state energy by the surface. Figure 7.7b shows the photoluminescence excitation (PLE) and PL spectra of HD-GQDs and LD-GQDs, respectively. HD-GQDs exhibit intensive blue emission which exhibits PL at 440 nm, and two PLE peaks at 295 nm (4.2 eV) and 375 nm (3.3 eV), respectively. In contrast, LD-GQDs exhibit ever-stronger blue emission of PL at 465 nm, while the PLE spectrum is peaked at 313 nm (3.96 eV), 382 nm (3.24 eV) and 420 nm (2.95 eV).
Figure 7.7 (a) UV-vis absorption spectra of as-prepared LD-GQDs and HD-GQDs, respectively, in water with arrows indicating the absorption peaks; (b) PL and PLE spectra of LD-GQDs and HD-GQDs; (c) Emission mechanism of the LD-GQDs and HD-GQDs.

The UV absorption and PLE spectra indicate the electron transitions of triple carbene from the highest occupied molecular orbital (HOMO) (σ and π orbital) to the lowest unoccupied molecular orbital (LUMO) as shown in Figure 7.7c.26,57-59 It can be found that the band gap of LD-GQDs is slightly smaller than that of HD-GQDs, not only for the reason of the larger average size but also of the less defects of LD-GQDs.42 The fluorescent quantum yields (FLQY) at 365 nm excitation were measured to be 2.4% and 1.8% for LD-GQDs and HD-GQDs, respectively, by using quinine sulfate as a standard (see Appendix Table A5.1). The higher FLQY for LD-GQDs may ascribe to their lower defective structures with sp³ carbon and fewer vacancy defect states in the basal plane in contrast with HD-GQDs.36,60 It should be noted that although the FLQY of the prepared GQDs are not as high as some previous work, the large-scale and fast processing can make up our GQDs to be the precursors for further modifications such as edge-sites amino doping to enhance their FLQY and optical performances.8,10,14,16,17,19,36

Figure 7.8 shows the PL spectra of as-synthesized HD-GQDs. The HD-GQDs exhibit strong excitation-dependent PL behavior (see Figure 7.8a and 7.8b), similar with previous observations. When excited at the wavelength range from 310-490 nm, the PL show red shifts from 428 to 528 nm, which is in accordance with the previous findings.8,9,12,24-26 This can also be proved by the bright blue, green
and red luminescence observed when excited by ultraviolet, blue and red light (see Figure A5.10). Figures 7c and 7d show the pH value dependence of PL intensity of HD-GQDs. When varying the pH value of HD-GQDs dispersion from 0.88 to 13.78, no obvious shifts of PL occurred. Instead, the shoulder of PL spectra around 440 nm increased. It should be mentioned that the intensity of PL increases nearly in a linear function of pH value (Figure 7.8d). The PL intensity varying with the pH value is attributed to the reversible protonation of free zigzag sites affected by the delocalized π-electron system.

In contrast, LD-GQDs show some differences in PL spectra as shown in Figure 7.9. Figures 7.9a and 7.9b show the grouping behavior of PL changing with the excitation wavelength. When excited at the wavelength range from 310-450 nm, the PL of LD-GQDs shows independence with the excitation wavelength, which is in accordance with the results reported in some work. But in the range of 450-530 nm, LD-GQDs exhibit excitation-dependent PL performances, which is different from previous works. Figure 9c-d shows the dependence of PL of LD-GQDs on pH value. When varying the pH value of LD-GQDs dispersion from 1.73 to 13.58, no obvious shifts of PL occur too. At low pH values 0-5, the PL intensity
only decreases slightly. However, the intensity of PL significantly decreases with further increasing pH value in the range of 5-14 (Figure 7.9d). This observation indicates that high OH\(^-\) content may induce PL quenched. This phenomenon is distinct from the most of previous results\(^{8,9,28,33-36}\) but agrees with that of GQDs synthesized by GO/DMF hydrothermal method\(^{11,12}\).

Figure 7.9 (a) PL spectra of LD-GQDs dispersed in water excited at different wavelengths from 350-530 nm; (b) The normalized PL spectra converted from (a); (c) PL spectra of LD-GQDs at different pH values excited at 370 nm; (d) dependence of PL intensity of LD-GQDs on pH value.

7.3.3 Bioimaging

Because of the excellent photoluminescence properties, stability, low cytotoxicity and biocompatibility, GQDs have been promoted to be promising for fluorescence probes in cell imaging\(^{10-12}\). To investigate possible application as fluorescence probe of as-prepared GQDs by the reported ultrasonic method, the fibroblast L929 cells of mouse were treated with the LD-GQD (Figure 7.10a-b) and HD-GQDs (Figure 7.10c-d) suspension (100 μg mL\(^{-1}\)) for 2 h, and irradiated under inverted microscope. The GQDs can be easily taken up by the fibroblast L929 cells. As shown in Figure 7.10, the profiles of fibroblast L929 cells under excitation (Figure 7.10b, d) are clearly shown by the PL of GQDs, which not only agree well with those viewed in the bright-filed imaging (Figure 7.10a and 7.10c), but also
reveal some detail profiles of the cell nucleus.

![Figure 7.10 The fibroblast L929 cells of mouse treated with LD-GQD (a-b) and HD-GQDs (c-d) suspension: (a, c) bright-field image; (b, d) confocal fluorescence photomicrograph under 488 nm excitation.](image)

In contrast to the control group of L929 cells without treatment with GQDs (see Figure A5.11), our GQDs exhibit good potential in visualization of living cells by photoluminescence.

In addition, the cytotoxicity of HD-GQDs was also evaluated by the MTT tests on the fibroblast L929 cells of mouse. Figure 7.11 shows the cell viability with increasing the concentration of GQDs in culture from 0-800 μg mL⁻¹. For the concentration of 0 and 200 μg mL⁻¹, the cell viability is almost 100%. From 200 to 800 μg mL⁻¹, the cell viability decreases almost linearly to 85%. Although trace NMP exists in the product, the MTT tests demonstrate that in the concentration range of 0–200 μg mL⁻¹, HD-GQDs have low cytotoxicity to the fibroblast L929 cells of mouse. Thus, the synthesized GQDs can be used as safe fluorescence nanoprobes for cell imaging.
7.4 Conclusion

In summary, a novel method for synthesizing GQDs by ultrasonic-assisted exfoliation of raw graphitic carbon materials in liquid-phase is developed. This method is fast, eco-friendly, low-cost, and can be used for mass production of GQDs in industry. The yield of GQDs reached a high value of 3.8 mg mL\(^{-1}\) in NMP solvent. The size and density of defects of GQDs can be tuned by selecting proper structures of graphitic carbon precursor materials. Two types of GQDs named high-defects GQDs and low-defects GQDs are synthesized from defects-rich acetylene black and low-density-defects nano-graphite, respectively. The GQDs exhibit different edge states and defects distributions, as well as different photoluminescence properties in quantum yield and PL changing with excitation wavelength and the pH value of GQDs dispersion. The performances of GQDs as fluorescence nanoprobes for bioimaging and cytotoxicity are also investigated. Our method offers a new strategy for large-scale synthesis of GQDs with different defects and structures, which can be used as the initials for further functionalization in the application of biology, electronic, energy and engineering.

References

GQDs-synthesis, optical properties and cell imaging


