Strongly green-photoluminescent graphene quantum dots for bioimaging applications†

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Strongly fluorescent graphene quantum dots (GQDs) have been prepared by one-step solvothermal method with PL quantum yield as high as 11.4%. The GQDs have high stability and can be dissolved in most polar solvents. Because of fine biocompatibility and low toxicity, GQDs are demonstrated to be excellent bioimaging agents.

Graphene has attracted tremendous attention because of its extraordinary mechanical, thermal, chemical and optical properties as well as potential applications.1,4 However, little effort has been made to provide a deep insight into the fluorescent property of “small” graphene, which is also called GQDs. Due to quantum confinement effect, the functionalities of quantum dots can be simply tailored by controlling the size. Consequently, quantum dots have potential applications in a new generation of detection, microelectronics, biomedicine and so on.5 In theoretical and experimental studies, quantum confinement and edge effects of “small” graphene (less than 10 nm) have shown to induce photoluminescence (PL).6,7 Additionally, it has been demonstrated that the isolated polyaromatic structures and passivated surface defects also contribute to the fluorescence of GQDs.8

Such “small” graphene can be labeled as a sort of fluorescent carbon-based nanomaterial,9 which contain fullerene,10 nanotubes,11 nanodiamond12 and carbon nanoparticles.13–21 These emerging carbon-based nanomaterials are applied in a multitude of exciting areas, ranging from medical diagnosis to catalysis and photovoltaics.9 Moreover, they are effective fluorescent probes for bioimaging due to their chemical inertia and nontoxicity in the physiological condition.11a,12,14a,15b Some papers concerning fluorescent graphene have been published. For example, fluorescent nano-graphene oxide can be prepared by the rate separation method;22 GQDs with blue photoluminescence have been prepared by cutting graphene with a hydrothermal route;23 PL can be induced in single layer graphene by appropriate oxygen plasma treatment24 or reducing graphene oxide (GO) thin film;25 luminescent GQDs have also been prepared by other methods.26 These materials are facing problems demanding urgent solution in several aspects such as fairly effective synthetic routes, high solubility for practical applications and clear PL mechanism.9–25 Thus far, the facile one-step synthetic route of GQDs in large scale is also highly desired.

Herein, we report a new method for the synthesis of strongly green-photoluminescent GQDs in large scale. A one-step solvothermal route from graphene oxide is implemented. The prepared GQDs possess strong fluorescence with PL quantum yields as high as 11.4%. Moreover, the GQDs can be dissolved in water and most polar organic solvents with no further chemical modifications. Due to the stable photoluminescence, low cytotoxicity, excellent solubility and biocompatibility, the GQDs are particularly eco-friendly and are demonstrated to be excellent probes for bioimaging.

Fig. 1 shows the Transmission Electron Microscopy (TEM) images of GQDs and their size distributions. The average diameters of GQDs are ~3.7 nm. Atomic Force Microscope (AFM) images of the GQDs (Fig. 1b) show that their average height is ~1.2 nm. Consequently, it can be concluded that most of the GQDs are single layered or bi-layered.22,28 In present experimental conditions, the decomposed temperature of GO measured by Thermo Gravimetric Analysis (TGA) was ~200 °C (Fig. S4†). This temperature is of significance for the synthesis process, because temperature less than 200 °C can not produce the GQDs effectively. During the reaction, dimethyl formamide (DMF) acted as the solvent as well as a weak reduction agent. The big GO was split and reduced simultaneously, and thus the GQDs were obtained.

The GQDs possess excellent solubility in water and most polar organic solvents. The as-prepared GQDs contain many chemical groups such as –OH, epoxy/ether, C=O and –CO–NR2 originated from decomposition of DMF (Fig. S5†), so they have high solubility without further chemical modifications. As a proof, the solubility of the GQDs was over 15 mg mL−1 in tetrahydrofuran (THF), acetone, DMF, dimethyl sulfoxide (DMSO), ethanol and water. These dispersions are stable at room temperature for at least six months.

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The full width at half maximum (FWHM) is about 100 nm, which approximates to that of the most reported carbon nanodots. Inset of Fig. 2a and b are the photographs of GQDs aqueous solution taken under visible and UV lights, the green fluorescent color is observed by the naked eye. Using 9,10-bis (phenylethenyl) anthracene as a reference, the PL quantum yield was measured to be 11.4%, which is higher than that of reported luminescent carbon nanomaterials.

**Fig. 2** The optical properties of the GQDs. (a) UV-vis absorption (ABS) spectra of the GQDs aqueous solution. (b) PL (at 375 nm excitation) spectra of the GQDs aqueous solution. (c) The excitation-dependent PL behavior of GQDs, when the GQDs were excited at wavelengths from 400 to 540 nm (20 nm interval), the PL peak shifts from 515 nm to 570 nm and the PL intensity decreases remarkably (dissolved in water). (d) Effect of solvents on the fluorescence of GQDs (at 375 nm excitation). From left to right, the solvents are THF, acetone, DMF, water, respectively, inset: photograph of the four dispersions taken under UV light.

All of the obtained GQDs exhibit excitation- and solvent-dependent PL behaviors. As they were excited at wavelengths from 400 to 540 nm, the PL peak shifted from 515 nm to 570 nm and the PL intensity decreased remarkably (Fig. 2c). This excitation-dependent PL behavior was extensively reported in fluorescent carbon-based nanomaterials, and it may result from optical selection of differently sized GQDs and surface defects of GQDs. The PL of the GQDs is also demonstrated to be sensitive to the species of solvent, as shown in Fig. 2d (at 375 nm excitation). The peak shifted from 475 to 515 nm in THF, acetone, DMF, water, respectively (inset of Fig. 2d is the photographs of them under a UV light). This solvent effect could be induced by solvent attachment or different emissive traps on the surface of GQDs.

The PL of the GQDs have outstanding stability compared to fluorescent dyes. No distinct photobleaching was found using hand UV lamp or Hg lamp with low power, but there is a decrease on PL intensity using 1000 W high pressure mercury lamp (Fig. S7a†). The fluorescence properties of the GQDs at different ionic strengths were monitored when increasing the concentration of KCl from 0 to 2.0 M. As shown in Fig. S7b, there were no changes in either the photoluminescent intensities or the peak characteristics. Another interesting phenomenon is the pH-dependent PL behavior (Fig. S7c†). PL intensities decrease in a solution of high or low pH, but remain constant in a solution of pH 4–8. Moreover, in a solution of pH over 12, the PL peak blue-shifts and the FWHM becomes narrow. This pH-dependent PL behavior is distinct from that observed in previously reported blue-luminescent GQDs, where their PL is quenched under acidic conditions (pH = 1). It also proves that the PL mechanism is not the same as emissive free zigzag sites.

For the PL mechanism of GQDs, it is speculated that both the quantum size effect and surface defects contribute to PL. Each GQD has some defects due to the incomplete reduction. FTIR have proved the –OH, epoxy/ether, –C=O and –CO–NR₂ (Fig. S5†); these groups cause the structural defects. Furthermore, from the XPS of the GO and GQDs (Fig. S8†), it is conjectured that there are three kinds of carbons: C=C, oxygenated C (which includes carbon–hydroxy groups C–OH, epoxy/ether groups C–O, carbonyl groups C=O and carbonyl groups O=C=O) and nitrous C. The percentage of C=C increases when GO was reduced to prepare GQDs (Table S2†). The structural changes were also proved through the zeta potential; the zeta potential of GO is about 52 mV, while the zeta potential of the GQDs is only 8 mV. As a result, the PL is controlled by size effect and surface defects simultaneously.

Apart from the bright fluorescence, high stability and low negative charge mentioned above, the GQDs also possess low cytotoxicity. Adding up to 400 µg of GQDs to 150 µL of culture medium (10⁴ cells) did not weaken the cell activity significantly, as shown by the MTT assay (Fig. 3a). GQDs uptake and bioimaging experiments were performed by the confocal fluorescence microscope as shown in Fig. 3b and c. Observation of the bright green area inside the cells indicates translocation of GQDs through the cell’s membrane (405 nm excitation). The excitation-dependent PL behavior of the GQDs gives rise to numerous visible results. When the
excitation light changes to 488 nm, green–yellow color is observed (Fig. 3d). Moreover, GQDs have different uptake behaviors for MC3T3 cell (Fig. S9f). These results indicate that GQDs can be used in bioimaging and other biomedical applications with high concentration.

In conclusion, strongly green fluorescent GQDs have been prepared via one-step solvothermal method from graphene oxide. The GQDs possess PL quantum yields as high as 11.4%. Moreover, due to the low cytotoxicity and excellent biocompatibility, GQDs are demonstrated to be an eco-friendly material as well as excellent biolabeling agents. In future work, the synthesis of more colorful fluorescent GQDs will be explored by means of a one-step method. Additionally, the PL mechanism of GQDs will be studied in full detail.

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Notes and references