Journal of Luminescence 197 (2018) 147–152

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Preparation of highly fluorescent sulfur doped graphene quantum dots for live cell imaging

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ARTICLE INFO

Keywords:
Sulfur doping
Graphene quantum dots
Cell bio-imaging

ABSTRACT

Herein, we reported a facile one-step hydrothermal strategy to synthesize stable, strong blue fluorescence and water-soluble sulfur doped graphene quantum dots (S-GQDs) with the citric acid (CA) and powdered sulfur (S) as the precursors. The results indicated that S atoms were successfully introduced into the structure of graphene, and sulfur doping indeed improved the intensity of blue emission of GQDs. The as-prepared S-GQDs, as the effective cell-imaging material, would easily penetrated into the cell membranes of Hela cell and exhibited relatively low cytotoxicity. When treated with the typical bacteria medium (*Staphylococcus aureus LZ-01 and Escherichia coli DH5α*), it also did not show the conspicuous antiseptic qualities. Therefore, the S-GQDs would have potential applications in the bio-imaging.

1. Introduction

Graphene quantum dots (GQDs) have attracted tremendous attention due to their excellent properties including high water solubility, low cytotoxicity, excellent biocompatibility and high resistance to photobleaching [1]. They also have wide range of applications in the field of photocatalysts, bio-imaging, ions detection, and electrochemical luminescence [2–5]. Generally, there are two approaches for preparation of GQDs, including “top-down” and “bottom-up” methods. Bottom-up methods have obvious advantages in adjusting the composition and physical properties of GQDs by choosing organic precursors or changing the reaction conditions. Bottom-up methods could also provide the effective way for doping heteroatoms into the structure of GQDs. As it known, doping other atoms could change the electronic density and effectively tune optical and electrical properties of GQDs [6]. Until now, a large number of researches have focused on the synthesis and properties of nitrogen doped graphene quantum dots (N-GQDs) [7]. Compared with the N-GQDs, sulfur doped graphene quantum dots (S-GQDs) also attracted much attentions for the reported excellent abilities, such as the electrocatalytic activity [8], hydrogen adsorption [9], high electrochemical capacity [10] rechargeable battery performance [11], and p-type semiconductor property [12]. It is of scientific interest and technical importance to investigate the optical and biological properties of S-GQDs.

However, sulfur doping is commonly realized by sulfur compounds (such as thiourea), which would be toxic with unpleasant smell [6]. Herein, we reported a facile and low cost hydrothermal route to synthesize S-GQDs by directly using the inexpensive and nontoxic powdered sulfur as the S source. The as-prepared S-GQDs exhibited strong blue fluorescence as well as the good water-solubility. And for bio-imaging measurements, the S-GQDs shows the low cytotoxicity and excellent cell-imaging for Hela cells. Moreover, when incubated with the typical bacteria medium (*Staphylococcus aureus LZ-01 and Escherichia coli DH5α*), it showed the weak antiseptic qualities. Therefore, the S-GQDs would have potential applications in the bio-imaging.

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https://doi.org/10.1016/j.jlumin.2018.01.028
Received 7 September 2017; Received in revised form 31 December 2017; Accepted 18 January 2018
Available online 06 February 2018

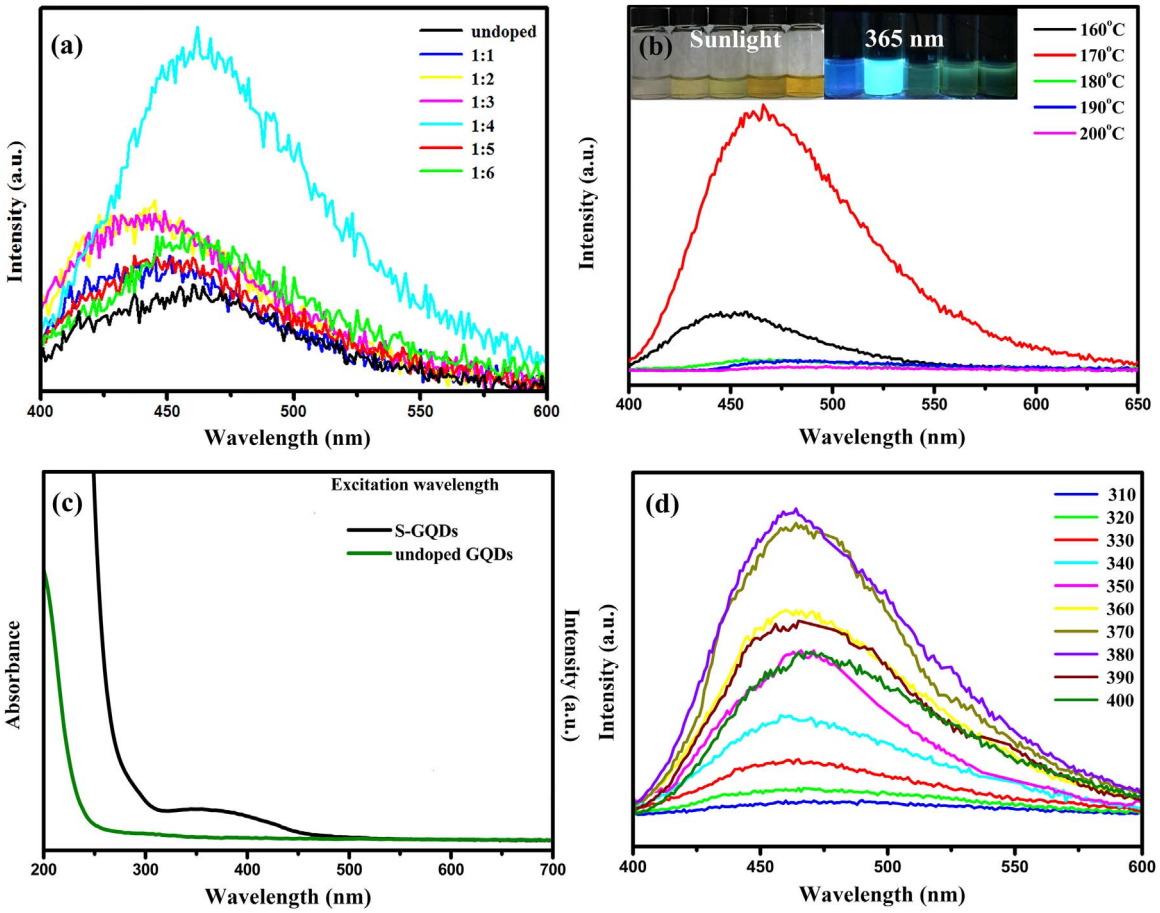

Fig. 1. (a) The emission spectra of S-GQDs under different ratios from1:1 to 1:6 (λ_{ex} = 365 nm). (b) The emission spectra of S-GQDs under synthesizing temperatures from 160 °C to 200 °C. The insets are optical images of S-GQDs aqueous solution under different temperatures and the diluted solution excited by 365 nm. (c) The absorption and the absorption of undoped GQDs. (d) The emission spectra of S-GQDs under different excitation wavelengths of 310–400 nm.

2. Experimental

2.1. Synthesis of S-doped GQDs

0.21 g (1 mmol) CA and 0.128 g (4 mmol) powdered S were dissolved into 5 ml deionized water. Then the solution was transferred into a 20 ml Teflon lined stainless autoclave. The sealed autoclave was heated to 170 °C in an electric oven and kept for additional 4 h. The final product was collected by adding ethanol into the solution and centrifuged at 5000 rpm for 5 min. The solid can be easily re-dispersed into water.

2.2. Cell imaging

Hela cells (the concentration of 2*105/hole) were plated on a 30-mm Petri dish in culture medium one day in advance for cell imaging. For in vitro study, cells were incubated in medium containing S-GQDs for 24 h, then treated with PBS for two times to wash the unabsorbed free dots. The whole system was imaged on laser scanning confocal microscopy (LSCM-710)

2.3. Methyl thiazolyl tetrazolium (MTT) assay

The cytotoxicity was assessed using the classic MTT assay with Hela cells. The liquid culture was poured to leave the adherent culture of Hela cells digested by E enzyme. When the digestion is finished, the cells were completely separated from the wall and dispersed evenly after percussed by 3 ml serum containing medium 1640. Using the counting plate, cell suspension with a suitable concentration certain volume were seeded into the 96-well plate at ~ 5 × 10^3 cells per well. The cells were incubated for 12 h in a humidified atmosphere at 37 °C under 5% CO_2. The S-GQDs solutions at various concentrations (10, 100, 200, 500 and 1000 μg/ml) were added to the wells and the cells were incubated for 72 h at the above cell culture conditions. A solution of 20 μL MTT (5 mg/ml) was then added into the wells and incubated for 4 h at 37 °C. Finally, the culture solution is dried and the dark blue formazan crystals were left at the bottom of the hole. Dimethyl sulphoxide (DMSO) was added to dissolve the formazan completely. The formazan concentrations were quantified using an enzyme-linked immunosorbent assay reader (ELISA FlexStation 3) to measure the absorbance at 570 nm. Parallel detection of 4 holes in each experiment were carried twice.

2.4. Experiment of inhibition zone

Put the beef Extract peptone agar medium which has been sterilized and cooled to about 50 °C respectively into two sterile plates, then placed the plates horizontally until the agar freeze. Use sterile straw to absorb 0.2 ml *Staphylococcus aureus LZ-01 and Escherichia coli DH5α* (from Ministry of Education Key Laboratory of Cell Activities and Stress Adaptations) bacteria suspension which have been cultivated for 18 h and add them into two sterile plates respectively, use sterile triangle coating bar to coat them evenly. Divided the even flat plate into 4 parts, marking each part with its reagent name. Use sterile tweezers to pick up the small round sterilized filter paper (D5 mm) and immersed them into the tube with aqueous solution of CA and S-GQDs in it, then soak the filter paper. (When removing the filter paper, guarantee that the amount of the solution in each paper basically consistent) With aseptic