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Ethylenediamine-assisted hydrothermal synthesis of nitrogen-doped carbon quantum dots as fluorescent probes for sensitive biosensing and bioimaging

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ABSTRACT

In this work, nitrogen-doped carbon quantum dots (N-CQDs) with high fluorescence quantum yield (46.2%) were successfully synthesized through one-pot hydrothermal carbonization approach assisted by ethylenediamine as surface passivation agent, wherein alanine acted as a single carbon source, ethylenediamine as the surface passivation agent. The obtained biocompatible N-CQDs could effectively label both the MCF-7 cell membranes and the nucleus, suggesting the potential application in biolabeling and bioimaging. Furthermore, the addition of dihydronicotinamide adenine dinucleotide (NADH) could dramatically quench the fluorescence of N-CQDs. Therefore, a novel protocol for NADH determination was established and its mechanism was discussed. This proposed fluorescence sensor displayed an enhanced performance for NADH determination with the linear range up to 80 μM and the detection limit down to 25.1 nM.

1. Introduction

In recent years, carbon quantum dots (CQDs) emerging as a new member of the carbon-based nanomaterials, similar to carbon nanotubes [1], fullerenes [2] and graphene [3], have drawn considerable attention in fluorescent sensor [4–10], biological imaging [11–15], drug delivery [16,17], photocatalysis [18,19] and LED device [20,21]. Simultaneously, many strategies including arc-discharge [22], laser-ablation [12,23], acidic oxidation [24–27], electrochemical synthesis [13,19,28], microwave synthesis [29–31], ultrasonic treatment [32,33] and hydrothermal carbonization [11,34–36] have been developed to synthesize CQDs since their serendipitous discovery by Xu group [22] in purifying single-walled carbon nanotubes.

Unfortunately, the preparations of CQDs through the above-mentioned methods usually involve expensive precursors, intricate processes or low quantum yields (QY). Therefore, it is necessary to develop a facile and cost-effective protocol for the CQDs synthesis. Since the nitrogen can provide the CQDs a new kind of surface state to enhance the fluorescence intensity [34,36], significant efforts have been devoted to synthesize the nitrogen-doped carbon quantum dots (N-CQDs). For instance, amino acids [29,37] or proteins [11] were selected as the ideal molecular precursors for synthesizing N-CQDs due to their abundance, cost effectiveness and biocompatibility. On the other hand, to dramatically improve the QY and biocompatibility of the as-synthesized CQDs, a number of post-treatment steps have been reported lately [12–15,30]. However, the post synthesis treatments, such as surface passivation scheme involving polyethylene glycol (PEG) [14,15] or polyethylenimine (PEI) [30], are time consuming and arduous. It is debatable whether or not the small organic compounds (ethylenediamine or other homologues) would be a promising alternative.

Meanwhile, as a fluorescent probe, high fluorescence quantum yield, low cytotoxicity and good biocompatibility are one of the most critical requirements for sensitive biosensing and bioimaging. For example, carbon dots as a non-enzymatic sensor were applied to blood glucose [6], dopamine [7], glutathione [8], tetracyclines [9] and so on. As a nontoxic and high-performance fluorescence imaging agent, the CQDs have been used for optical imaging in vitro [11–15,38] and in vivo [14,39–41]. Nevertheless, some of these recently reported studies still show negative results due to poor properties of CQDs. Much work is still needed to exploit these nanomaterials in sensitive biosensing and bioimaging.

Herein, we developed a facile and economic synthetic approach for the preparation of N-CQDs via hydrothermal carbonization alanine and ethylenediamine for the first time. The formation of N-CQDs and the surface passivation by ethylenediamine were

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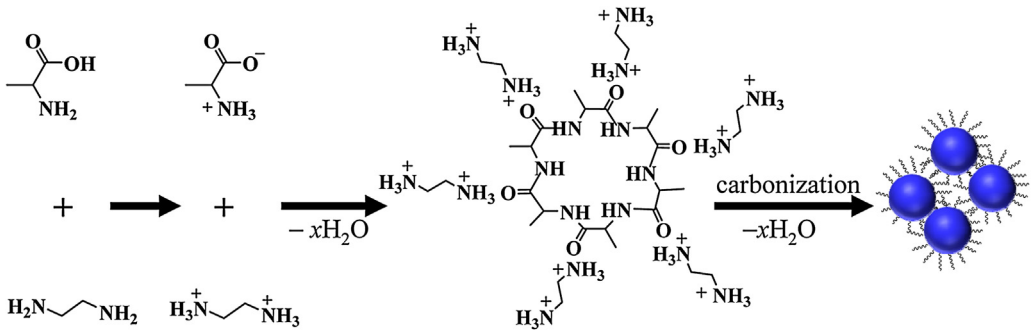

Fig. 1. Processing diagram for the synthesis of ethylenediamine-passivated CQDs.

accomplished simultaneously in one pot. The spectral properties and biocompatibility of the N-CQDs are studied in detail. Furthermore, a novel N-CQDs sensor for NADH is proposed based on the effective fluorescence quenching of N-CQDs and the quenching mechanism is discussed as well.

2. Experimental

2.1. Chemicals and materials

Alanine (Ala) and ethylenediamine were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), nicotinamide, D-biotin, adenosine 5′-triphosphate disodium salt hydrate (ATP), glutathione, folic acid and vitamin $B_1/B_2/B_6$ were obtained from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Sunshine Biotechnology Company (Nanjing, China). All other chemicals were of analytical grade and used without further purification. Phosphate buffer solution (PBS) was 0.1 M K_2HPO_4 and KH_2PO_4 and its pH was adjusted with H_3PO_4 or KOH solutions. Twice-distilled water was used throughout the experiments.

2.2. Preparation of N-CQDs

The N-CQDs were prepared by a modified hydrothermal carbonization protocol [34,35]. In a typical one-step synthesis, 2.673 g alanine was dissolved into 30.0 mL of ultrapure water, followed by adding 500 μL ethylenediamine under stirring. Then, the solution was transferred into the Teflon-lined autoclave chamber (50.0 mL) and heated to 200 °C for 6 h. After the reaction, the reactors were cooled to room temperature. The product, which was brown-black and transparent, was dialyzed in a dialysis bag (1000 MWCO) for 24 h. Finally, the solution was dried at 45 °C by rotary evaporators to obtain solid powders. The major steps and mechanism involved in the synthesis of N-CQDs are illustrated in Fig. 1.

2.3. Characterization

The morphology and size of N-CQDs were analyzed using high-resolution transmission electron microscopy (HR-TEM, Philips TECNAI-12) under the accelerating voltage of 300 kV. The samples were prepared by the deposition of 10 μL N-CQDs aqueous solutions on a copper grid coated with ultra-thin films of carbon, while the solvent was removed by evaporation in air at room temperature. The crystalline structure and the surface functional properties of N-CQDs were investigated by X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific Co., USA). Infrared spectra were measured on a pressed KBr pellet employing a TENSOR Model 27 FT-IR spectrometer. The fluorescence and the absorption spectra were measured by fluorescence spectrophotometer (Edinburgh Analytical Instruments, FLS920) and UV–vis spectrophotometry (UV-3600, Shimadzu, Japan), respectively. Fluorescence lifetime measurements were conducted with the time-correlated single-photon counting technique on the combined steady-state and lifetime spectrometer (Edinburgh Analytical Instruments, FLS920).

2.4. Quantum yields measurements

The quantum yields (ϕ) of the N-CQDs were calculated following slope method [35], where the quinine sulfate (0.1 mol/L H_2SO_4 as solvent; QY = 0.54) were chosen as standards. Specifically, we compared the absorbance (several values, less than 0.1 at excitation wavelength) and the corresponding integrated photoluminescence intensity of N-CQDs aqueous solutions with quinine sulfate references at their optimal excitation wavelengths, respectively. Then, the integrated photoluminescence intensity against the absorbance was plotted and a linear regression was attained, where the slope of the curves could be obtained. Finally, we used the equation as follows:

$$\phi_x = \phi_{st}\left(\frac{K_x}{K_{st}}\right)\left(\frac{\eta_x^2}{\eta_{st}^2}\right)$$

where ϕ is the QY, K is the slope determined from the curves, η is the refractive index of the solvent (both are 1.33). The subscript st and x are denoted to quinine standard and N-CQDs aqueous solutions, respectively.

2.5. Cytotoxicity testing of the N-CQDs

The cytotoxicity of the N-CQDs was evaluated by the MTT assay. Briefly, human lung cancer A549 and human breast cancer MCF-7 cells were first cultured with high-glucose DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution in an incubator (37 °C, 5% CO_2) until cell counts were 4×10^3 in each well. Then, the culture medium was replaced with 100 μL of DMEM containing the N-CQDs at different doses (0, 10, 25, 50, 100, 200, 400 μg/mL) to seed for another 24 h. After that, 20 μL of 5 mg/mL MTT solution was added to each cell well and the cells were further incubated for 4 h, followed by removing the culture medium with MTT, 150 μL of DMSO was added. The resulting mixture was shaken about 10 min at room temperature and the optical density (OD) was measured at 490 nm with a microplate reader. Finally, the cell viability was estimated as the following equation:

$$\text{Cell viability}\,(\%) = \left(\frac{\text{OD}_{\text{Treated}}}{\text{OD}_{\text{Control}}}\right) \times 100\%$$

where $\text{OD}_{\text{Control}}$ was obtained in the absence of N-CQDs, $\text{OD}_{\text{Treated}}$ was obtained in the presence of N-CQDs.