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Rapid microwave synthesis of N-doped carbon nanodots with high fluorescence brightness for cell imaging and sensitive detection of iron (III)

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ABSTRACT

We rapidly prepared N-doped photoluminescent carbon nanodots (CNDs) with the one-step microwave irradiation method using diammonium hydrogen citrate as the carbon source. The as-prepared CNDs possessed quasispherical morphology and a high quantum yield of about 26.8%, which was higher than the CNDs obtained by most other microwave-assisted methods. Moreover, the luminescent CNDs could be efficiently uptaken by BGC-823 cells and CT26.WT cells, and exhibited low cytotoxicity and favorable biocompatibility, making them suitable candidates for cell imaging. In addition, the CNDs could be utilized for Fe^{3+} ions sensitive detection with a detection limit of 180 nM.

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1. Introduction

Fluorescent probes with prominent optical properties could be used for future cell biology and biomedical applications, such as biodetection, bioimaging, and disease diagnosis [1,2]. Fluorescent carbon nanodots (CNDs) are members of the family of carbon nanomaterials, and have a lateral size of less than 10 nm. CNDs have attracted tremendous attention in the fields of biolabeling, biomedicine, sensors, photocatalysis, optoelectronic devices, and so on [3–6]. Compared to commonly used probes, such as traditional organic dyes and semiconductor quantum dots (QDs), CNDs are promising candidates, because of their aqueous solubility, high photostability, robust chemical inertness, easy functionalization, excellent photobleaching resistance, low toxicity, and good biocompatibility [7–10]. There are various methods that can be used to prepare CNDs, such as the hydrothermal method [11], laser ablation [12], electrochemistry [13], ultrasonic vibration [14], microwave radiation [15], and so on. However, current some methods for preparing CNDs are complex, or quantum yields of prepared CNDs are generally low, which greatly limit its application in the fields of cell imaging and biodetection as a fluorescent probe.

At present, microwave radiation has been regarded as the most effective, inexpensive, and rapid method to synthesize fluorescent CNDs, because it is easy to perform, does not require much equipment or many instruments, and saves time [10]. The CNDs passivated by organic solvent can effectively improve CNDs' fluorescence quantum yield, the possible mechanism of which is that the passivation agent may fill the surface defects of CNDs, or the present of surface energy traps may lead to visible luminescence of CNDs [12]. Doping is also an effective method for improving quantum yield of CNDs, because of doping creating n-type and p-type carriers, which change the electronic structures of CNDs [8]. The synthesis and passivation of CNDs could be completed in one step during microwave irradiation, making it an economical and environmentally friendly method for the large-scale production of highly fluorescent CNDs.

Herein, we rapidly prepared the N-doped fluorescent CNDs with the one-step microwave irradiation method, as shown in Fig. 1, using diammonium hydrogen citrate as the carbon source containing nitrogen and poly(ethylene glycol) (PEG-400) as the passivation reagent, without adding any other acid or alkaline solvent. The as-prepared CNDs had quasispherical morphology with a high quantum yield of about 26.8%, which was higher than the CNDs obtained by most other microwave-assisted methods [15–18]. In addition, the N-doped CNDs could be utilized for Fe^{3+}

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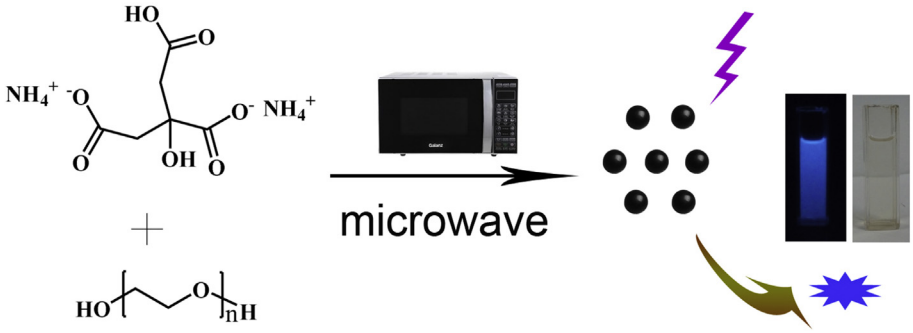

Fig. 1. Schematic illustration for microwave irradiation synthesis of N-doped CNDs.

ions sensitive detection, with a detection limit of 180 nM, and for cell imaging as label-free fluorescent nanoprobes. Additionally, the one-step synthesis process of CNDs is facile, efficient, and cost-effective, and could potentially allow for large-scale production.

2. Experimental

2.1. Materials

Diammonium hydrogen citrate, disodium hydrogen citrate, PEG-400, and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Citric acid, ammonium oxalate, and ammonium acetate were purchased from Shanghai Aladdin Reagent Co., Ltd. (China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich. The Roswell Park Memorial Institute (RPMI)-1640 medium was supplied by Gibco Life Technologies (Shanghai, China). The semipermeable membranes were obtained from Shanghai Yuanye Biological Technology Co., Ltd. The BGC-823 and CT26.WT cells were purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). Other chemicals were of analytical grade and used as received. Deionized water with a resistivity of 18.1 MΩ cm was used throughout the experiments.

2.2. Synthesis of N-doped CNDs

In a typical microwave-assisted synthesis procedure, 0.4 g of diammonium hydrogen citrate and 10 mL of PEG-400 were added to 10 mL of deionized water to form a transparent solution, and then heated in a domestic microwave oven (750 W, Galanz, China) for 6 min. The color of the solution gradually changed from transparent and colorless to wine red, and then to dark brown. When the reaction mixture was cooled down to room temperature, the CNDs solution was dialyzed with the semipermeable membrane (MWCO = 500) for 48 h. The solution was freeze-dried after dialysis, weighed, dissolved in deionized water, and used for cytotoxicity and cell imaging measurements.

2.3. Characterization

The size and morphology of N-doped CNDs were observed using high-resolution transmission electron microscopy (HRTEM) on a JEM-2100 (JEOL, Japan). For TEM imaging, 10 μL of CNDs suspension was deposited on a carbon-coated copper grid and dried under room temperature. Fourier transformed infrared (FTIR) spectra were acquired over the range of 400–4000 cm^{-1} on a Nicolet 380 FTIR spectrometer (Thermo, USA). For IR analysis, a sample was dispersed in KBr and pressed into a sheet with appropriate pressure. A total of 32 scans were accumulated with a resolution of 4 cm^{-1} for each spectrum. X-Ray photoelectron spectra (XPS) were obtained using a Thermo Scientific ESCALAB 250Xi with Multitechnique Surface Analysis. The photoluminescence spectra were collected on a Cary Eclipse fluorescence spectrophotometer (Varian, USA) equipped with a Xe lamp as the light source. UV–vis absorption spectrum of CNDs was acquired on a U-3900 spectrophotometer (Hitachi, Japan), using a 1 cm path length cuvette.

2.4. Quantum yield measurements

The quantum yield(φ) of the N-doped CNDs was estimated by comparing the integrated luminescence intensities (excited at 360 nm) and the absorbance values (at 360 nm) of the CNDs with that of the quinine sulfate in 0.1 M H_2SO_4($\varphi = 0.54$). Specifically, the absorbance values of CNDs and quinine sulfate were measured at 360 nm. Fluorescence spectra of CNDs and quinine sulfate were also recorded at an excitation wavelength of 360 nm to obtain the integrated luminescence intensity, which is the area under the photoluminescence emission curve in the wavelength range from 380 to 700 nm. The quantum yield of the CNDs was calculated using the following equation:

$$\varphi_x = \varphi_{st} \times \frac{I_x}{I_{st}} \times \frac{A_{st}}{A_x} \times \frac{\eta_x^2}{\eta_{st}^2},$$

in which the subscripts *st* and *x* denoted quinine sulfate standard and CNDs, respectively, φ and I were the fluorescence quantum yield and integrated emission intensity, and A and η were the optical density and refractive index (both are 1.33). To minimize the reabsorption effects, we kept the absorbance at 360 nm in a 1 cm cuvette below 0.05.

2.5. Effects of pH and metal ions on the photoluminescence of CNDs

Here, 40 μL of synthesized CNDs (10 mg mL^{-1}) was added in 3200 μL of phosphate buffers with different pH values. The corresponding photoluminescence spectra of CNDs at different pH were then acquired upon excitation at 370 nm. Various metal ions, including Na^+, K^+, NH_4^+, Ag^+, Pb^{2+}, Cu^{2+}, Ba^{2+}, Fe^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, Y^{3+}, Eu^{3+}, and Fe^{3+} in aqueous solutions (10 mM) were first prepared. Then, 40 μL of synthesized CNDs (10 mg mL^{-1}) was diluted with 3200 μL of each metal ion solution. The mixed solutions were incubated for 5 min. Then, the fluorescence spectra of CNDs in the absence and presence of various metal ions were recorded upon a 370 nm excitation.

2.6. MTT cell viability assay

BGC-823 and CT26.WT cells were cultured in a RPMI-1640

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