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Naked oats-derived dual-emission carbon nanodots for ratiometric sensing and cellular imaging

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ABSTRACT

We report a green and facile strategy for fabrication of dual-emission carbon nanodots (CDs), and demonstrate their applications in ratiometric sensing and cellular imaging. Dual-emission CDs have been prepared by pyrolysis and microwave treatment of naked oats, providing a novel way for the production of dual luminance CDs without the request of tedious synthetic methodology or the use of toxic/expensive solvents and starting materials. Their intriguing dual fluorescence behavior is observed when the excitation wavelength is between 250 and 310 nm. The well-resolved dual emission bands manifest excitation and temperature dependence. The obtained CDs were applied as a ratiometric fluorescent sensing platform for precise and quantitative detection of Al^{3+} ions and pH values, and as optical nanoprobes for cellular imaging.

1. Introduction

Fluorescent nanoparticles have a tremendous impact on the advancement of a wide range of fields including electronics, photonics, energy, catalysis, and medicine [1]. To date, typical photoluminescent (PL) particles have been developed from compounds of lead, cadmium, gold, silver and silicon [2–5]. But these materials also have raised concerns over potential toxicity, environmental harm and high cost [5,6].

Carbon nanodots (CDs), fascinating fluorescent nanoparticles, are generally defined as carbon nanomaterials with sizes below 10 nm and considered to consist of an amorphous or crystalline core with predominant sp^2 carbon and an oxidized carbon surface with carboxyl group [7]. By virtue of their low cost, high aqueous solubility, low photobleaching, non-blinking, low toxicity, and excellent biocompatibility, CDs have gradually become superior alternatives to other fluorescent nanoparticles [8]. These distinct benefits of CDs make them great promise for a variety of practical applications, such as bioimaging [9], chemical sensor and biosensor [10–12], drug delivery [13], gene delivery [14], dye sensitizers [15], catalysis [16,17], fluorescent ink [18], and mimetics peroxidase [19]. Currently, CDs are typically synthesized by two types of strategies: top-down and bottom-up routes. Top-down approaches involve laser ablation or electrochemical oxidation of graphite [20,21], electrochemical treatment of multiwalled carbon nanotubes [22], and chemical oxidation of commercially activated carbon [23]. Bottom-up methods include pyrolysis [24,25], wet oxidation [26,27], hydrothermal synthesis [28–31], and microwave-assisted synthesis [18,32] with all sorts of carbon precursors, such as plant leaf [24], glycerol [25], hair fiber [26], tire soot [27], Bombyx mori silk [28], soy milk [29], saccharides [30,31], citric acid [18], and amino acid [32], etc.

During the past few years, many attempts have been made toward lowering the cost, simplifying operation, improving quantum yield (QY), or realizing larger-scale production of CDs. However, tuning the PL behavior of CDs has been less explored. Giannelis et al. [33] for the first time developed dual emission N-doped CDs by the controlled pyrolysis of citric acid and ethanolamine (1:3 molar ratio) at different temperatures and also elaborated the PL mechanism of the CDs arising from the excitation state of the core domains and the surface chemistry. Since then, dual-emission CDs, momentous supplement to single fluorescence CDs, have attracted wide attention because of their potential applications in chemical sensing and biological tracing. Wang et al. [34] proposed an ingenious strategy for fabrication of dual emission cross-linked fluorescent CDs by mixing acetic acid, water, and

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diphosphorus pentoxide, and Tang et al. [35] attributed dual fluorescence bands of as-prepared CDs to the emission from the core and the surface states. Xiong et al. [36] reported novel long-chain cationic surfactant (nonylphenol polyethylene glycol quaternary ammonium salt (NPEQ)) coated CDs through ionic interaction and discovered that the aqueous solution of obtained CDs presented distinct dual emission peaks ascribing to a partial ionization of NPEQ molecules from CDs. Hu et al. [37] applied an effective approach to prepare dual emission N-doped CDs by the pyrolysis of PAN (Poly Acrylonitrile)@PMMA (Poly Methyl methacrylate) core–shell nanoparticles at different temperatures and clarified the PL mechanism of the CDs originating from the amide groups (or imine groups) of the surface and the amorphous carbonaceous core. Nevertheless, all these methods suffer from some drawbacks like the requirement of tedious synthetic methodology or the use of toxic/expensive solvents and starting materials. Thus the production of dual-emission CDs toward green and facile fabrication is a challenging but worthy issue.

In this contribution, we present a green and facile approach to obtain dual-emission CDs from naked oats by pyrolysis and microwave treatment for the first time. Compared with the previous works, our devised method avoids the request of tedious synthetic methodology or the use of toxic/expensive solvents and starting materials. Remarkably, the as-synthesized CDs display excitation and temperature tunable dual luminescence and favorable stability under UV radiation or long-term storage. Coupled with the characteristic PL properties and enhanced stability, the as-synthesized CDs have been successfully utilized to ratiometric fluorescent sensing of Al^{3+} ions and pH values, and confocal fluorescent microscopic images of human renal clear cell carcinoma (786-0) cells.

2. Materials and methods

2.1. Materials

$AlCl_3$, $BaCl_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$, $FeCl_3$, $HgCl_2$, KCl, $MgCl_2$, $MnCl_2$, NaCl, $NiCl_2$, and $ZnCl_2$ were purchased from Beijing Chemical Corp. (Beijing, China). NaH_2PO_4 and Na_2HPO_4 were purchased from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). Naked oats were purchased from local market (Taiyuan, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Solarbio (Beijing, China). Distilled deionized (DDI) water was obtained from a Millipore Milli-Q-RO4 water purification system with a resistivity higher than 18 MΩ cm^{-1} (Bedford, MA, USA).

2.2. Synthesis and PL enhancement of CDs

CDs were synthesized by pyrolysis of naked oats. In a typical run, a crucible loaded with 5 g of naked oats was transferred into a muffle furnace, and pyrolyzed at 400 °C for 2 h. After cooling down to room temperature, the black products were mechanically ground to fine powders. 1 g of the resultant sample was dispersed in 5 mL DDI water and magnetically stirred to form a black solution. The CDs aqueous solution were collected by removing larger particles through centrifugation at 12 000 rpm for 10 min.

PL enhancement of CDs was achieved by further microwave treatment. Typically, the obtained CDs aqueous solution was treated in a domestic microwave oven (700 W) for 12 min.

2.3. Characterization

Transmission electron microscopy (TEM) study was carried out in a JEOL JEM-2100 instrument operating at an accelerating voltage of 200 kV. Samples for TEM measurements were prepared by placing a drop of colloidal solution on carbon-coated copper grid and then dried at room temperature. The size distribution of CDs was performed by counting over 100 particles. Fluorescent photographs of CDs under UV light of 365 nm were operated with ZF-2 ultraviolet analyzer for three purposes from Shanghai City Anting electronic instrument factory. Fourier Transform infrared (FTIR) spectra were recorded on Nicolet Magna 550 spectrometer using a resolution of 4 cm^{-1}. The sample with 1 mg diluted by KBr (ratio 1:200) was pressed into the disc. X-ray photoelectron spectroscopy (XPS) data were obtained with an AXIS ULTRA DLD electron spectrometer from Shimadzu company using 300 W Al Kα radiation. The base pressure was about 3×10^{-9} mbar and the binding energies were referenced to the C_{1s} line at 284.6 eV from adventitious carbon. UV–vis absorption spectra were recorded on a Puxi TU-1901 UV–vis absorption spectrophotometer (China). Steady-state fluorescence measurements were performed on a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). Fluorescence lifetime measurements were performed on an Edinburgh FLS920 spectrometer (Edinburgh, UK). A nanosecond hydrogen flash lamp was used as the excitation light source in the determination of Fluorescence lifetime.

2.4. QY measurements

The relative QY (Φ) of the CDs was calculated using the equation of $\Phi_x = \Phi_{std} I_x A_{std} \eta_x^2 / (I_{std} A_x \eta_{std}^2)$. The optical densities were measured on Puxi TU-1901 UV–vis absorption spectrophotometer. In the equation, I_x and I_{std} are the fluorescence intensities of the CDs and the standard, and A_x and A_{std} are the optical densities (OD) of the CDs and the standard, respectively. Quinine sulfate in 0.1 M H_2SO_4 was chosen as a standard with a quantum yield $\Phi_{std} = 0.54$ at 360 nm. η_x and η_{std} are the refractive index of the CDs and the standard, respectively. The absorbencies of all the samples in 1.0 cm cuvette were kept under 0.1 at the excitation wavelength to minimize re-absorption effects.

2.5. Fluorescence assay of Al^{3+} ions

The detection of Al^{3+} ions was performed at room temperature in 10 mM phosphate buffered saline (PBS) (containing 150 mM NaCl, pH 5.0). In a typical run, 30 μL of CDs dispersion (1.6 mg/mL) was added into 300 μL of PBS, followed by the addition of Al^{3+} standard with various concentrations. The fluorescence emission spectra were recorded after reaction for 1 min at room temperature. The sensitivity and selectivity measurements were conducted in triplicate.

River water sample was treated by ultrafiltration with 10 kD ultrafiltration membrane, spiked with standard Al^{3+} ion at different concentration levels, diluted within the working linear range, and analyzed with the proposed method and inductively coupled plasma atomic emission spectrometry (ICP-AES).

2.6. Fluorescence assay of pH values

The detection of pH values was performed in 10 mM PBS (containing 150 mM NaCl) with various pH values. In a typical run, 30 μL of CDs dispersion (1.6 mg/mL) was added into 300 μL of PBS with various pH values. The fluorescence emission spectra were recorded after reaction for 1 min at room temperature. The measurements were conducted in triplicate.

2.7. MTT assay

For the cell cytotoxicity text, 786-0 cells were first plated on a Costar 96-well tissue-culture cluster and cultured at 37 °C with 5% CO_2 in air for 3 h to adhere cells onto the surface. The well