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Novel and green synthesis of high-fluorescent carbon dots originated from honey for sensing and imaging

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ABSTRACT

An innovative and green strategy to synthesize carbon dots (CDs) with a quantum yield (QY) of nearly 19.8% has been successfully established for the first time. Subsequently, the possible fluorescence (FL) mechanism was elucidated by fluorescence, UV–vis, high resolution transmission electron microscope (HR-TEM), Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) analyses. Significantly, not only the precursor of CDs and whole synthesis procedure was green, but also the CDs obtained here exhibited various advantages including high fluorescent QY, excellent photostability, non-toxicity and satisfactory stability. Additionally, the CDs were employed for assaying Fe^{3+} based on direct interactions between Fe^{3+} and –COOH, –OH and $–NH_2$ of CDs, resulting in aggregations that facilitate to quench their fluorescence. The decrease of fluorescence intensity permitted detections of Fe^{3+} in a linear range of 5.0×10^{-9}–1.0×10^{-4} mol/L, with a detection limit of 1.7×10^{-9} mol/L at a signal-to-noise ratio of 3, suggesting a promising assay for Fe^{3+}. Eventually, the CDs were applied for cell imaging and coding, demonstrating their potential towards diverse applications.

1. Introduction

Carbon nanomaterials, mainly including carbon nanotubes, fullerenes graphene, and carbon nanofilms, have been playing critical roles in various fields such as material science (Prato, 1997), biochemistry and biomedicine (Yang et al., 2009). Recently, carbon dots (CDs), emerging as a new class of fluorescent nanomaterials, have attracted considerable interest. Based on carbon skeleton structure, carbon dots usually exist in the size of less 10 nm, showing excellent properties such as ease of preparation (Jia et al., 2012; Liu et al., 2007), satisfactory fluorescent performance, low cytotoxicity and biocompatibility (Baker and Baker, 2010) owing to their specific nanometer dimension. Thus, CDs have been considered as a satisfactory candidate for biosensing (Wang et al., 2010), catalysis (Cao et al., 2011), and imaging (Wei et al., 2012; Zhao et al., 2011; Zhou et al., 2012). In the past few years, CDs were generally synthesized by two major methods. One way was known as top down, consisting of electrochemical oxidation (Zhao et al., 2008; Zhou et al., 2007), acidic oxidation (Dong et al., 2010), arc discharge (Xu et al., 2004) and laser ablation (Hu et al., 2009). Hydrothermal (Zhu et al., 2012), microwave (Salinas-Castillo et al., 2013) and ultrasonic (Zhuo et al., 2012), serving as another way defined as bottom up, have been normally applied to synthesize CDs. Nevertheless, these methods exhibited several drawbacks such as tedious steps, toxic reagents, special equipments, strong acid (alkali) or high temperature and high costs, leading to their limitations for applications (Sahu et al., 2012). Therefore, exploring new methods for synthesizing CDs are still desired.

As being well known, Fe^{3+} served as an essential component of heme groups and one of abundant transition metals in human biological systems (Von Drygalski and Adamson, 2013). Currently, there exist various techniques for quantification of Fe^{3+} including flame atomic absorption spectroscopy (Ajlec and Stupar, 1989), inductively coupled plasma mass spectrometry (Huang and Lin, 2001), atomic absorption/emission spectrophotometry (Wu et al., 2009), atomic fluorescence and UV spectrometry (Kok and Wild, 1960). However, most of these methods required sophisticated and expensive instrumentation and/or complicated sample preparation procedures and were time consuming (Lee et al., 2011; Wang et al., 2012; Xiang and Tong, 2006). In contrast, fluorescent sensors offered a simple, low-costly approach for assaying metal ions in biological and environmental samples.

In this study, a simple, economical, and green method for preparing water-soluble CDs has been established with a quantum yield of 19.8%. Importantly, this strategy for producing CDs by one-step treatment of honey under low-temperature heating was reported for the first time (Fig. 1). In addition, both the precursor of CDs and the synthesis procedure are substantially environment friendly, leading to their biocompatibility and more extensive applications. Subsequently, we

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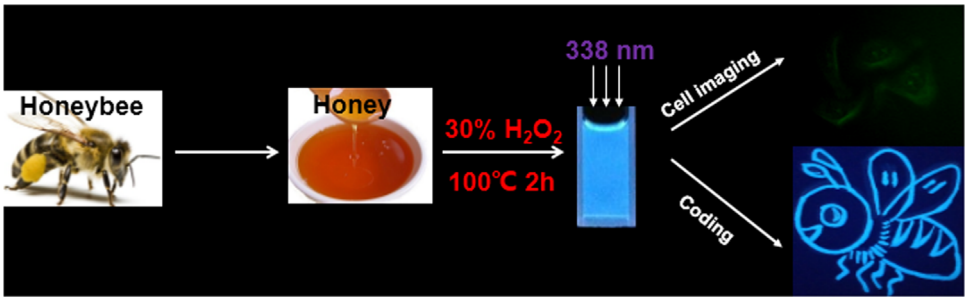

Fig. 1. Schematic illustration of synthesis of CDs and cells imaging (top) and coding (bottom).

applied the CDs for sensitive and selective detection of Fe^{3+} based on direct interactions between Fe^{3+} and –COOH, –OH and $–NH_2$ of CDs (Chinoporos, 1962; McIlwee et al., 2008; Moon et al., 2010), resulting in aggregations that facilitates the quenching of their fluorescence. Again, the practicability of this strategy has been validated by assaying Fe^{3+} in human blood samples. Meanwhile, the CDs were employed for cell imaging, coding and preparing fluorescent powder, suggesting their potential to broaden avenues for meaningful applications and commercial purpose.

2. Experimental

2.1. Chemicals and materials

Honey was bought from Yonghui Supermarket (Chongqing, China). All the metal ions (Hg^{2+}, $Fe^{2}+$, Pb^{2+}, Ag^{+}, Ca^{2+}, Co^{2+}, Mn^{2+}, Sr^{2+}, Zn^{2+}, K^{+}, Na^{+}, Cu^{2+}) and trihydroxymethyl aminomethane (Tris) were obtained from Shanghai Sangon Biotechnonlogy Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4), 30% Hydrogen peroxide (30% H_2O_2), potassium dihydrogen phosphate (KH_2PO_4), citric acid and sodium citrate, glacial acetic acid (HAc), phosphoric acid (H_3PO_4), boric acid (H_3BO_3), sodium hydroxide NaOH), sodium chloride (NaCl), ammonium chloride (NH_4Cl) were purchased from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Ultrapure water, 18.25 MΩ cm, produced with an Aquapro AWL-0502-P ultrapure water system (Chongqing, China) was employed for all experiments.

2.2. Instrumentation

All fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with excitation slit set at 5 nm band pass and emission at 5 nm band pass in 1 cm × 1 cm quartz cell. Meanwhile, UV/vis absorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). The high resolution transmission electron microscopy (HR-TEM) images were taken using a TECNAI G2 F20 microscope (FEI, America) at 200 kV. Elemental and functional groups analysis were obtained by an ESCALAB 250 X-ray photoelectron spectrometer and a Fourier transform infrared spectrometer (Tokyo, Japan), respectively. The quantum yields were obtained by using an Absolute PL quantum yield spectrometer C11347 (Hamamatsu, Japan). The powder of CDs obtained by lyophilisation in PiloFD8-4.3V (SIM, USA). Photographs of cells were taken with an Olympus fluorescence microscope 1 × 71 (Tokyo, Japan). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was used to measure the pH values of the aqueous solutions and a vortex mixer QL-901 (Haimen, China) was used to blend the solution. The thermostatic water bath (DF-101s) was purchased from Gongyi Instrument Co., Ltd. (Henan, China).

2.3. Synthesis of CDs

Basically, the green CDs were synthesized for the first time here. In brief, 2.0 g honey was added into 8 mL ultrapure water at the beginning. After stirring for mixing, this solution was introduced with 2 mL of 30% H_2O_2. Then the mixture was transferred into a 25 mL Teflon-lined stainless-steel autoclave. After heating at 100 °C for 2 h, the autoclave was cooled to room temperature, and this aqueous solution was adjusted to neutral with 2 M NaOH followed by filtered with 0.22 μm filter membrane to remove the larger product. Finally, the fluorescent carbon dots were collected by dialysis against deionized water though a dialysis membrane (1000MWCO) for 24 h. The powder of CDs was obtained by lyophilisation, and furhter dissolved in ultrapure water with the final concentration of 1 mg/mL. The CDs prepared here were stable for 3 months while stored in the dark at 4 °C.

2.4. Detection of Fe^{3+}

Firstly, 40 μL CDs (1 mg/mL), 40 μL Britton–Robinson (BR) buffer (50 mM, pH 6.0) and an appropriate volume of Fe^{3+} working solution or sample solution were successively pipetted into a 1.5 mL vial. Subsequently, these solutions were diluted to 400 μL with Milli-Q purified water, and followed by vortex-mixed thoroughly. After reacting at 35 °C for 10 min, the mixtures were subjected to fluorescence measurements. Finally, interferences originated from other metal ions were investigated individually in the presence of CDs prepared here.

2.5. Preparation of blood samples

Fresh human blood samples were originally collected from three healthy volunteers of Southwest University Hospital. In brief, 1 mL blood was initially dispensed in 5 mL of Red Blood Cell (RBC) lysis buffer, and incubated at room temperature for 15 min. Then, the lysed samples were centrifuged at 10,000 rpm for 10 min, and the supernatant were collected. Next, 0.2 mL 20% ascorbic acid was introduced into the above solutions to oxidize Fe^{2+} to Fe^{3+} for further analysis. Finally, the prepared samples were used for detections of Fe^{3+} according to the general procedure without additional special treatment.

3. Results and discussion

3.1. Characterization of CDs

To characterize this synthesized CDs, the maximum excitation and emission spectra of synthesized CDs were initially recorded as 338 nm and 420 nm (Fig. 2A) respectively, and the fluorescent properties of the CDs solution were subsequently investigated. The CDs aqueous solution emitted obvious blue fluorescence