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Nitrogen and phosphorus co-doped carbon dots derived from lily bulbs for copper ion sensing and cell imaging

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ABSTRACT

A straightforward, rapid, and environmentally-friendly technique was developed for the synthesis of nitrogen and phosphorus co-doped fluorescent carbon dots (CDs) with the use of lily bulbs (LB) as raw materials. The as-synthesized LB-CDs showed high stability in aqueous solution and high fluorescence with a quantum yield (QY) of 17.6%. The LB-CDs were then used as a fluorescence sensor for Cu^{2+} ion detection based on the Cu^{2+}-ion-stimulated fluorescence quenching of LB-CDs. The synthesized LB-CDs demonstrated exceptional selectivity and sensitivity to Cu^{2+} ions, with a limit of detection of 12.8 nM and a linear detection range of 0.05–2.0 μM. The synthesized LB-CDs were also applied for the detection of Cu^{2+} ions in real water samples. Moreover, the LB-CDs exhibited low cytotoxicity, which is suitable for fluorescence-based analysis and cell imaging.

1. Introduction

Copper is considered an important transition metal for human beings and has an important role in biological systems. Copper is mostly found in natural and environmental water, for instance, river water, lake water, tap water, seawater, and drinking water [1,2]. Nevertheless, copper is highly toxic at high concentrations with long-term contact with the human body. Excessive copper concentration can cause numerous neurodegenerative ailments, such as Alzheimer's disease, prion disease, and Wilson's disease [3]. In accordance with the US Environmental Protection Agency, the maximum limit of Cu^{2+} in drinking water should be 20 μM [4]. So, monitoring the environmental copper ions concentrations with elevated sensitivity and selectivity is important. Although different analytical approaches, including electrochemical sensors, inductively coupled plasma mass spectrometry, potentiometric sensors, atomic absorption spectroscopy, and atomic fluorescence spectroscopy [5–9], can be applied to detect copper ions with high sensitivity, their applications are limited by high costs, time-consuming process, intricate specimen development, and improper real-time inspection.

Fluorescent carbon dots (CDs) have attracted the interest of researchers because of their promising uses in different fields ranging from drug delivery [10], sensing [11–13], optoelectronic apparatus [14] to imaging [15]. In comparison with traditional organic fluorescent dyes and heavy-metal-based quantum dots, CDs have been emphasized because of their simple synthesis, exceptional water solubility, low toxicity, elevated photostability, affordability, and extraordinary biocompatibility [16–18]. In particular, on the basis of their powerful and adjustable photoluminescence (PL) characteristics, CDs are applied to detect heavy metal ions [19]. Currently, some natural items that contain nitrogen, for instance, papaya [20], winter melon [21], and konjac flour [22], were used as precursors for the fabrication of nitrogen-doped CDs with the application of hydrothermal treatment. However, the nitrogen-doped CDs developed from these affordable and abundant precursors did not attain high quantum yield (QY) (typically < 10%), resulting in problems during practical use. It has been reported that heteroatom-doped carbon nanomaterials are motivating high research preferences that are capable of productively tuning their inherent properties, including optical features and local and surface chemical properties [23,24]. Thus, the search for a method for the synthesis of heteroatom-doped and high PL CDs is recommended. Li et al. proposed a rapid and effective microwave-assisted technique for the purpose of synthesizing phosphorus and nitrogen co-doped CDs from N-phosphonomethyl aminodiacetic acid and ethylenediamine for fluorescent cell imaging [25]. Gong's group synthesized phosphorus and nitrogen co-doped carbon quantum dots with brilliant green PL by mixing glucose, 1, 2-ethylenediamine, and concentrated phosphoric acid (H_3PO_4) [26]. Nevertheless, all these mechanisms take a long time and are not eco-friendly, confining their broad use. Accordingly, establishing a straightforward and environmentally-friendly technique to obtaining highly fluorescent doped CDs remains a challenging task.

Most natural substances have intricate components, and CDs developed from these substances normally have different surface groups with unique properties. To the best of our knowledge, the synthesis of

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phosphorus and nitrogen co-doped CDs from natural sources has never been reported. Therefore, the development of rapid and affordable approaches for the synthesis of nontoxic phosphorus and nitrogen co-doped fluorescent CDs from natural sources still continues to be a challenge. Lily bulbs (LB) have abundant carbon, nitrogen, oxygen, phosphorus, and hydrogen components because of the presence of carbohydrates, proteins, lipids, and amino acids. Accordingly, we expect that this natural substance should have a potential for the synthesis of phosphorus and nitrogen co-doped CDs.

In this study, we have established an exceptionally green and affordable strategy to fabricate elevated luminescent LB-CDs through the application of one-pot microwave-assisted treatment of LB. The synthetic LB-CDs exhibit elevated fluorescence with QY of 17.6%, with extraordinary selectivity and sensitivity to Cu^{2+}. The detection limit for Cu^{2+} is as low as 12.8 nM, which shows that this substance is an appropriate candidate for the ecological detection of Cu^{2+}. The findings from the calculation of Cu^{2+} in river water provide evidence for our supposition and indicate its promising use in practical applications. Meanwhile, LB-CDs were applied to cell imaging, which indicates their capability in the biomedical fields.

2. Experimental

The materials used, the characterization methods, and the cell toxicity tests are shown in the supporting information (SI).

2.1. Preparation of LB-CDs

LB-CDs were synthesized through a microwave-assisted approach that used LB as carbon precursor. Briefly, 12 g of newly cut LB was shifted to a domestic 800 W microwave oven, followed by heating for 6 min in an autoclave. Deionized water (20 mL) was added to the reaction mix after cooling down to room temperature in a natural manner. The suspension was centrifuged (10000 rpm, 15 min), followed by passing through a 0.22 μm syringe-driven filter and dialysis against deionized water with the help of a dialysis membrane (1000 MWCO) for 48 h. Finally, a translucent brown aqueous solution that contains LB-CDs was lyophilized to obtain dry LB-CDs.

2.2. Fluorescence QY calculation

The determination of the QY of LB-CDs was made in accordance with a developed mechanism. Briefly, quinine sulfate in 0.1 M sulfuric acid (QY = 54%) was selected as the standard. Absorbances in the 1.0 cm fluorescence curette were maintained under 0.1 at the excitation wavelength of 370 nm to minimize the reabsorption effects. The QY of LB-CDs was calculated with the application of the following equation:

$$Q = Q_R \times (I/I_R) \times (A_R/A) \times (n/n_R)^2,$$

where Q indicates the QY, I indicates the calculated integrated fluorescent emission intensity, n indicates the refractive index of the solvent, and A denotes the optical intensity at excitation wavelength. The subscript R refers to the standard.

2.3. Detection of Cu^{2+}

The fluorescent detection of Cu^{2+} ions was conducted at room temperature in distilled water. In this specific test, an appropriate quantity of Cu^{2+} ions was added into 2 mL of acetic acid–sodium acetate buffer solution (pH = 4) that contained LB-CDs. The fluorescence emission spectra were recorded after the 3 min reaction at room temperature. Confirmation of the selectivity of Cu^{2+} ions was conducted through the addition of various stock solutions of metal ions rather than Cu^{2+} ions in the same manner.

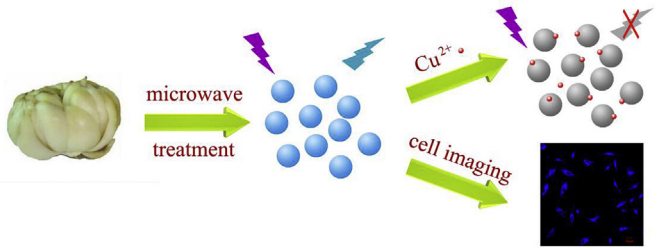

Fig. 1. Schematic of the preparation of LB-CDs and their application to Cu^{2+} detection and cell imaging.

2.4. Cell imaging

Human lung adenocarcinoma A549 cells were cultured in high-glucose Dulbecco's modified Eagle's medium that contained 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10% fetal bovine serum. In the duration of propagation, human lung adenocarcinoma A549 cells were dispersed in 24 duplicate wells and incubated at a temperature of 37 °C in a 5% CO_2 incubator for 24 h. Subsequently, the culture source was substituted with a fresh source that contained LB-CDs at the density of 1 mg mL^{-1}, followed by the incubation of A549 cells for 2 h. The cells were washed three times using phosphate-buffered saline and imaged with an inverted Olympus IX51 fluorescence microscope.

3. Results and discussion

3.1. Characterization of LB-CDs

As presented in Fig. 1, LB-CDs were prepared using LB as carbon precursor through a plain-microwave-assisted treatment. The size allocation and morphology of the attained LB-CDs were characterized using TEM. As indicated by the TEM images, LB-CDs are homogeneous, with particles spread in a spherical shape (Fig. 2a). The diameter of the obtained LB-CDs ranged between 1.34 nm and 5.23 nm, with the median size of 3.15 nm (Fig. 2b). The Fourier transform infrared (FTIR) spectrum was obtained to identify the functional groups of LB-CDs, as presented in Fig. 3a. The distinctive peak at 3277 cm^{-1} is ascribed to the stretching vibrations of the –OH and H_2N– groups, which ensure the dispersibility and constancy of LB-CDs in water. The peak at 2921 cm^{-1} is attributed to the stretching vibration of the C–H group. The peak situated at 1583 cm^{-1} stems from the N–H flexing vibrations. The peak centered at 1380 cm^{-1} is attributed to the C–N stretching vibrations. The peak located at 1251 cm^{-1} stems from the C–P stretching vibrations. The peak at 1027 cm^{-1} is ascribed to the C–O stretching vibrations. X-ray photoelectron spectroscopy (XPS) analysis was also conducted to examine the surface functional groups and element states of LB-CDs. The existence of C, N, O, and P in the synthesized LB-CDs is confirmed by XPS (Fig. 3b). The C1s XPS spectrum exhibits four kinds of carbon atoms (Fig. 3c), as follows: C = C/C–C having a binding energy of 284.5 eV; C–P having a binding energy of 285.2 eV; C = O having a binding energy of 287.9 eV; and C–N having a binding energy of 286.3 eV. The N1s spectrum exhibits two peaks at 399.8 and 401.5 eV (Fig. 3d) that are attributed to the C–N–C and surface H_2N– groups, respectively. The O1s spectrum exhibits two peaks at 531.8 and 532.8 eV (Fig. 3e) that are ascribed to the C–N–O/P–O and C–OH groups, respectively. The P2p spectrum (Fig. 3f) sheds light on the existence of P–O (133.1eV), P–C (134.0 eV), and $P2p_{3/2}$ (133.4ev). FTIR and XPS characterizations indicate that nitrogen and phosphorus have been successfully doped into LB-CDs.

3.2. Spectral properties

As depicted in the UV–vis spectrum (Fig. 4a), the LB-CDs solution