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Bioimaging Applications of Excitation-Independent Fluorescence Carbon Dots

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Abstract

Herein, carbon dots (CDs) were synthesized via a simple method and applied in bioimaging. The CDs display excitation-independent fluorescence emission and exhibit upconversion PL, providing further tissue penetration and greater sensitivity as the effect of the autofluorescence of biological bodies is minimized and cellular imaging. This is the first time that excitation-independent carbon dots is prepared via a truly facile approach without the need of external heating or additional energy input. These results indicate that the CDs have excellent potential to be applied in biomedical and clinical analysis.

Keywords: bioimaging; carbon dots; excitation-independent fluorescence

Fluorescence imaging is an important tool in biomedical applications that uses visible light and NIR spectra to analyze the distribution of molecules of interest in cells and tissues(1). Solubility determine whether a fluorescence material can be used in the body. Many fluorophores suffer poor water solubility and often require an extra bioconjugation step to render it soluble in water. Semiconductor nanometer materials, for instance, require surface coating with polymers or ligands to become soluble in water(2), and their constituents have proven to be toxic.

Carbon Dots (CDs) are winning wide attention in recent years(3–6) Due to their superior water solubility, good biocompatibility, excellent optical features, and simplicity in modification, CDs have demonstrated various promising applications, such as drug delivery, biochemical sensing, and bioimaging, etc(7–10). Thus, tremendous efforts have been made on the simple preparing of CDs, such as the combustion of carbon fibers(11) with nitric acid; electrochemical oxidation of graphite(12); microwave irradiation of acetic acid(13) and hydrothermal treatment of sucrose(14); These approaches have many disadvantages, from

complicated experimental set-ups, the need of strong acids, and significantly high energy consumption through to the difficulty in preparing long-wavelength(including yellow light region) and upconversion emissive CDs. They are not conducive for developing a green synthesis method to obtain CDs with good optical features. Among the excellent optical properties of CDs, the excitation-dependent fluorescent emission have been extensively studied. On the other hand, however, few examples of excitation-independent CDs with upconversion luminescence have been reported.

By taking into account the above considerations, a simple, facile and energy-efficient pathway to attain yellow luminescent CDs is presented. This approaches is energy, labour and cost efficient. Indeed, the only steps required are mixing glucose, L-cysteine and sodium hydroxide solutions, leaving them alone at room temperature for 24h and then dialysing, drying to obtain fluorescent CDs. Scheme 1 illustrates the process of synthesizing CDs. Briefly, glucose (1 g), L-cysteine (0.33g) are dissolved in 20 ml of 1 M sodium hydroxide solution. After being incubated at room temperature for 24 hours, the solution turn brown, which suggests the form of CDs.

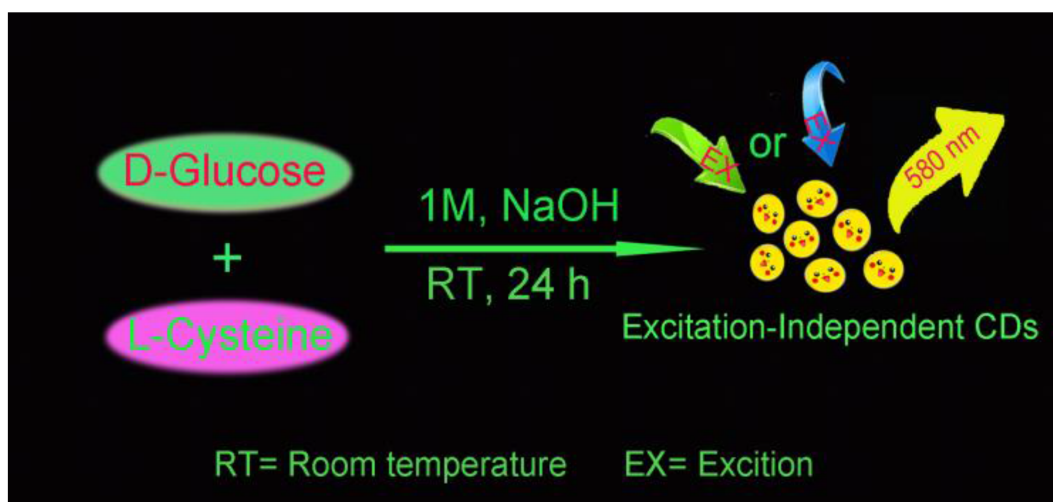


Figure 1. Schematic illustration for the formation process of the CDs.

Transmission electron microscopy (TEM) is performed to testify the as-prepared CDs (Fig. 2A). The CDs display uniform nanoparticles with an average diameter of 6.4 nm. In addition, as shown in Fig. 2B, X-ray diffraction (XRD) pattern is employed to characterize the crystal nature of the CDs with a peak at about 22.6 degree, which could be attribute to the (002) facet of graphitic carbon.

The Fourier transform infrared (FTIR) spectrum of the CDs is shown in Fig. 2C. The characteristic peaks suggest the aromatic skeleton of C=C, aromatic amine, carboxyl, hydroxyl groups C-S and C=S are present on the surface of the CDs. The hydrophilic groups (such as amine, carboxylic groups) endow these CDs with excellent water-solubility. As shown in Fig. 2D, the UV-vis absorption spectra of the CDs aqueous solution are described. The CDs exhibit two absorption peak at 334nm and 420nm, the unusually broad and strong UV-vis absorption band, especially in the visible-light region, suggesting the presence of

multiple electronic absorption transitions.

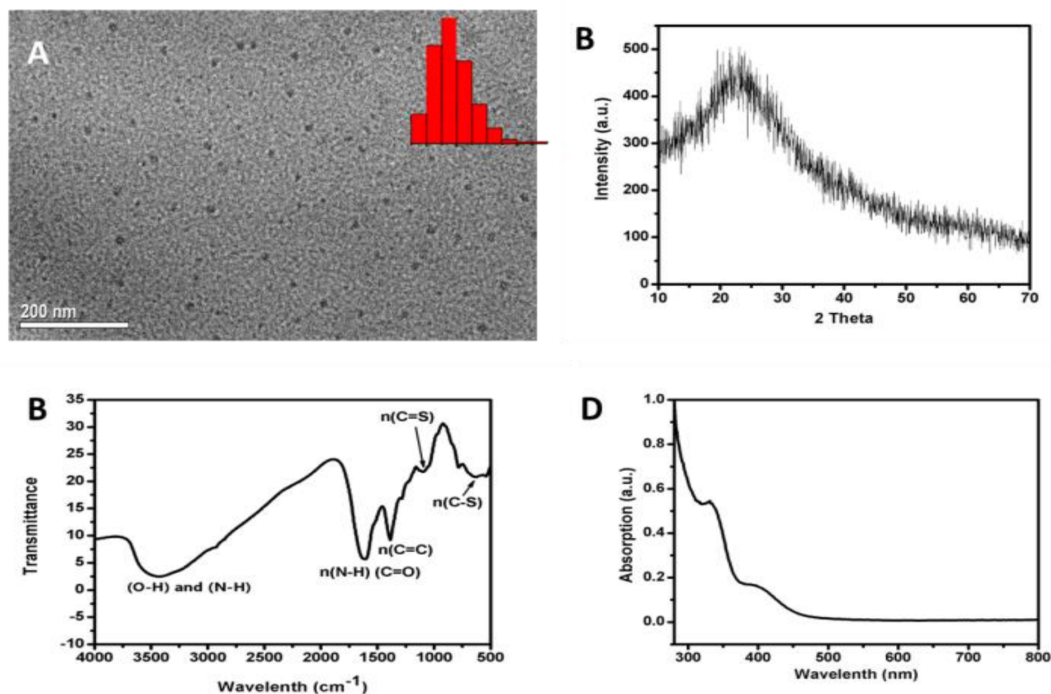


Figure 2. TEM image, inset: size distribution(A); XRD spectrum(B); FTIR spectrum(C) and UV-Vis absorption spectrum(D) of CDs.

Photoluminescence properties of the successfully obtaining CDs are investigated. Surprisingly, the CDs display unusually optical properties: their photo-luminescence emission wavelength is at around 580 nm, showing no significant shift as altering the excitation wavelengths from 400 to 520 nm together with from 830 to 900 nm (see Fig. 3A, C). This phenomenon is quite different from the usually reported excitation-dependent emissions of CDs synthesized by conventional methods (15, 16). It is generally believed that the different sizes and multiple surface emissive trap status could result in adjacent vibrational relaxation together with inter-system crossing of excited electrons, and then will trigger fluorescence emission with the corresponding energy (17, 18). In this work, it is hypothesized that glucose as a carbon precursor with a simple structural component, could avoid the generation of complex by-products during the reaction process. Furthermore, as the stabilizer, L-cysteine could promote the formation of fluorescent CDs with homogeneous surface states (19). As a result, the fluorescence emissions of these CDs are irrespective of the excitation wavelengths. Excellent optical properties and remarkable biocompatibility endow the water dispersible CDs with great potentials as a bioimaging reagent.

To test the stability of the as-prepared CDs, the effects of extreme pH, high ionic strengths, long time storage and continuous illumination on the fluorescence intensity of CDs are investigated (Fig. 3B, 3D, 3E and 3F). There is no obvious change in both the peak position and the fluorescence intensity, indicating a superior ionic-, time- and photo-

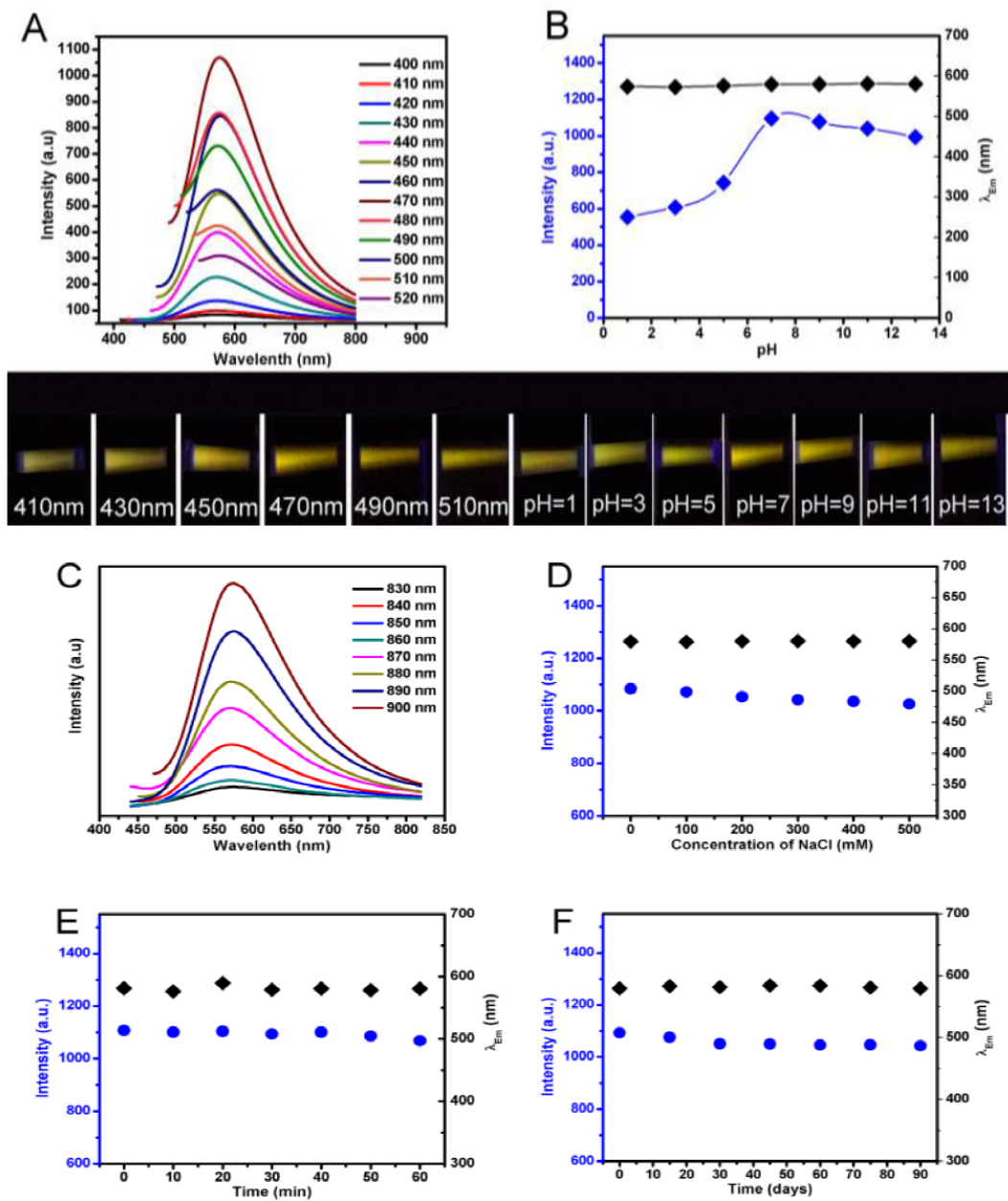


Figure 3. Fig. 3 (A, C) Fluorescence spectra of CDs at different excitation wavelengths and their corresponding optical photographs. (B) Fluorescence emission spectra of CDs at various pH values excited by 470 nm blue light and corresponding optical photographs. Stability study of as-synthesized CDs against change in the (D) ionic strength, (E) UV radiation (365 nm), and (F) storage time at room temperature.

stability of CDs. However, the CDs demonstrate tunable photoluminescence according to the pH value of their aqueous dispersions. As illustrated in Fig. 3B, a significant increase of the photoluminescence intensity is recorded within pH 0 – 7 and then decrease slowly at

high pH values of 7–13. The corresponding optical photographs of CDs aqueous dispersions at different pH values are also captured (Fig. 3). All of these results indicate that the as-synthesized CDs have great potential for biological sensing and imaging.

In order to explore the important feature of as-prepared CDs in the field of bioimaging. The cells are incubated with the CDs for 1 h at 37°C without any further functionalization for cell imaging applications. Followed by image acquisition via fluorescence microscopy under excitation by blue light, the cell-internalized CDs emit remarkable yellow colors (Fig. 4F, 4H). The cell imaging result indicates that the CDs can be applied in clinical and biomedical analysis.

Fig. 4 (A–B) Fluorescence microscopy images of the cells: (B) incubated with CDs for 1 hour. (A) Bright-field images of (B).

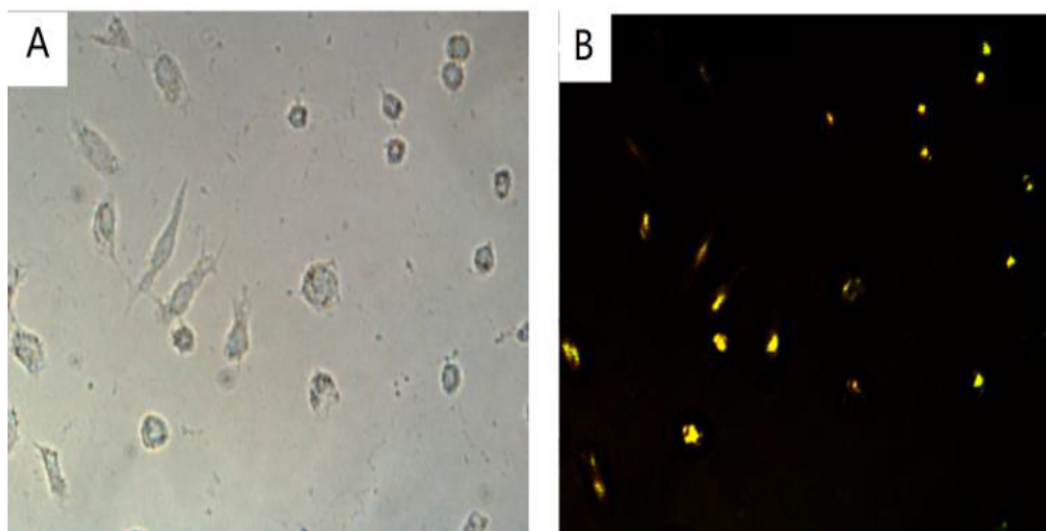


Figure 4. The Operation Process of Stretcher Patient Transport

Conclusion In summary, we have synthesized yellow and upconversion luminescent CDs via a material, energy and labour efficient approach. The presented method is very simple, facile, low-cost and free of complicated equipment. These CDs possess uniform morphology, excitation-independent fluorescence emission. Furthermore, the CDs show unique yellow fluorescence, unusual broad and strong UV-vis absorption, very good photostability and remarkable biocompatibility, which is obviously different from previous reports. Moreover, imaging of human lung adenocarcinoma cells further suggest that they can bring promising applications. Furthermore, the CDs exhibit upconversion fluorescence, allowing further tissue penetration and extending the scope of the usefulness of CDs in bioimaging(20).

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