Simple Approach to Synthesize Amino-Functionalized Carbon Dots by Carbonization of Chitosan

Xin Liu, Jinhui Pang, Feng Xu & Xueming Zhang

Carbon dots (CDs) as a new series of fluorescent nanomaterials have drawn great attention in recent years owing to their unique properties. In this paper, a simple carbonization approach to synthesize amino-functionalized CDs was developed by using chitosan as the carbon precursor. The as-prepared CDs possessed desirable amino function group on their surface and exhibited bright luminescence with absolute quantum yield (QY) of 4.34%, excitation-, pH-dependent and up-conversion fluorescence behaviors. Furthermore, we have investigated the cytotoxicity and biocompatibility of the as-prepared CDs, which demonstrated that the as-prepared CDs have the potential applications in biosensing, cellular imaging and drug delivery.

Results and Discussion

Physical structure of as-prepared CDs. The transmission electron microscopy (TEM) and atomic-force microscopy (AFM) images (Fig. 2) illustrated that the as-prepared CDs were quasi-spherical and monodisperse with size distribution between 1–6 nm in diameter. Besides, the high resolution transmission electron microscopy (HRTEM) image (inset of Fig. 2(a)) clearly showed the lattice spacing of 0.18 nm, which might be assigned
to the (102) facet of graphitic carbon\textsuperscript{15}. The X-ray diffraction (XRD) pattern of chitosan and as-prepared CDs were displayed in Fig. 3(a). The spectrum of chitosan showed distinct diffraction peaks at 11.24 and 19.96\(^\circ\), corresponding to the amorphous peak and crystalline peak, separately\textsuperscript{22}. After carbonization, the crystallinity of chitosan diminished and the spectrum of as-prepared CDs showed a broad peak at 2\(\theta\) = 22.48\(^\circ\), similar to the graphite lattice spacing, which was attributed to highly disordered carbon atoms\textsuperscript{23,24}. Additionally, the Raman spectrum of as-prepared CDs (Fig. 3(b)) revealed that although the fluorescence background signal was very strong, a relatively strong D band at 1378 cm\(^{-1}\) and G band at 1580 cm\(^{-1}\) could be obviously observed, and the ratio of intensities of I\(_D\)/I\(_G\) was calculated to be 1.15, indicating the presence of more defects or amorphous carbon as compared with sp\(^2\) hybridization in as-prepared CDs\textsuperscript{25–27}, which coincided with the HRTEM and XRD results.

**Chemical structure of as-prepared CDs.** The Fourier transform infrared (FT-IR) spectra of chitosan and as-prepared CDs were presented in Fig. 4(a). The basic characteristic peaks of chitosan appeared at 3438 cm\(^{-1}\) (O-H and N–H stretching vibrations), 2920 and 2875 cm\(^{-1}\) (C-H stretching vibration), 1643 and 1602 cm\(^{-1}\) (N-H bending vibration), 1493–1245 cm\(^{-1}\) (C=C, C=N and C=C–O groups) and 1165–992 cm\(^{-1}\) (C-H bending vibrations of the pyranose ring)\textsuperscript{14}. However, for the as-prepared CDs, the adsorption of O-H and N-H stretching vibration at 3401 cm\(^{-1}\) were decreased. Meanwhile, the C-H bending vibrations associated with pyranose ring in chitosan were completely disappeared. These differences between chitosan and as-prepared CDs might be
ascribed to the decomposition of the chitosan chain and pyranose ring during the carbonization process. Notably, the bands at 2930 and 1615 cm\(^{-1}\) corresponded to N-H bending vibration of amino group increased\(^2\). The temperature programmed desorption of CO\(_2\) (CO\(_2\)-TPD) profile of as-prepared CDs (Fig. 4(b)) suggested a distinct desorption of CO\(_2\) centered around 76 °C, similar to previous reports for amino-modified solids\(^2\), which might be due to the interaction between amino-functionalized CDs and CO\(_2\) via a chemisorption mechanism. The general formula of interaction between the functional amine and the CO\(_2\) molecules results in the formation of ammonium carbamates under anhydrous conditions can be seen in Equation (1)\(^3\):

\[
2R - NH_2 + CO_2 \rightleftharpoons R - NHCOO^- + R - NH_3^+ \quad (1)
\]

Also, the zeta-potential of as-prepared CDs was positive when the pH was below 6.67, indicating the existence of amine groups on the surface of as-prepared CDs together with the FT-IR and CO\(_2\)-TPD results\(^3\).

X-ray photoelectron spectroscopy (XPS) measurement was carried out to further investigate functional groups presented on the surface of as-prepared CDs. The whole XPS survey spectrum of the as-prepared CDs (Fig. 4(c)) exhibited three obvious peaks at 284.81, 399.33, and 532.3 eV, which were attributed to C 1s, N 1s and O 1s, respectively. The C 1s XPS spectrum (Fig. 4(d)) can be further deconvoluted into four carbon states at 284.82, 285.40, 286.12 and 288.79 eV, which were attributed to C-C/C≡C, C-N, C-O and C=O/N/C=O respectively.
and might result in a series of emissive traps between $\pi$ and $\pi^*$ states of $C=\mathrm{C}$. The O 1s spectrum (Fig. 4(e)) showed two peaks at 532.01 and 533.17 eV, which were assigned to the C=O and C-O bands, respectively. Furthermore, the N 1s spectrum (Fig. 4(f)) revealed an apparent peak at 399.83 eV, which was associated with N-H band. The results from XPS spectra showed that the presence of hydrophilic functional groups on the surface of the as-prepared CDs without further modification. And these groups made as-prepared CDs aqueous dispersible and made a significant contribution to their optical properties.

Optical properties of as-prepared CDs. Although the fluorescence emission mechanisms remain debatable, two classes of fluorescence emission mechanisms have been proposed. The first class mechanism is related to the bandgap transitions caused by conjugated $\pi$-domains, while the second class mechanism arises from the existence of multiple surface defects. The Ultraviolet-visible (UV-Vis) absorption and photoluminescence (PL) spectra of as-prepared CDs were investigated as shown in Fig. 5. From UV-Vis absorption, it was obviously that a strong UV-Vis absorption peak located at 261 nm, which typically assigned to the $\pi-\pi^*$ transition of aromatic $sp^2$ domains from the carbon core. As shown in the PL spectra, when excited at the maximum excitation wavelength of 310 nm, the as-prepared CDs exhibited a strong PL peak at 390 nm. The aqueous solution of as-prepared CDs was nearly transparent under visible light, while it displayed strong blue fluorescence under UV light (365 nm) excitation. (inset, Fig. 5)

Furthermore, the phenomenon of excitation dependent was clearly observed in Fig. 6(a). The emission spectra were sensitive to the excitation wavelength and the emission peak varied from 370 nm to 490 nm as the excitation wavelength increased from 250 nm to 430 nm. When the excitation wavelength increased from 250 to 310 nm, the emission peak become stronger and redshift. When excited from 310 to 430 nm, the emission peak kept redshift with remarkably decreased intensities. The excitation-dependent and redshift optical properties were similar to previous studies. As shown in Fig. 6(b), the up-conversion fluorescence emission phenomenon was observed that when as-prepared CDs were excited by longer wavelength (450–550 nm), shorter wavelength (400 nm) was emitted, which may be caused by the two or multiphoton active process. Figure 6(c) illustrated that the optical responses of the as-prepared CDs with pH values ranging from 1 to 13. It was clearly that PL emission wavelength of as-prepared CDs were stable in the pH range of 5 to 9. In strong acidic condition, the emission wavelength evolved to the longer wavelength, while moved to shorter wavelength in strong basic solution, implying that the as-prepared CDs were very sensitive to acid/base solvent. These tunable fluorescence emission properties of as-prepared CDs were particular attractive for sensing, in vivo bioimaging and synthesizing novel photocatalysts.

Fluorescence decay curve and the exponential fitting curve of as-prepared CDs were showed in Fig. 6(d). The fitting formula is:

$$R(t) = B_1 e^{(-t/\tau_1)} + B_2 e^{(-t/\tau_2)} + B_3 e^{(-t/\tau_3)} + B_4 e^{(-t/\tau_4)}$$  \hspace{0.5cm} (2)

where $t$ is time, $B_1$, $B_2$, $B_3$, and $B_4$ are fractional intensities, $\tau_1$, $\tau_2$, $\tau_3$, and $\tau_4$ are fluorescence lifetime. The parameters generated from iterative reconvolution of the decay with the instrument response function (IRF) were listed in the inset of Fig. 6(d). And according to formula:

$$\tau_{\text{ave}} = \frac{B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3 + B_4 \tau_4}{B_1 + B_2 + B_3 + B_4}$$  \hspace{0.5cm} (3)

we calculated the amplitude-weighted average fluorescence lifetime ($\tau_{\text{ave}}$) of as-prepared CDs to be 6.0921 ns, which suggested that the as-prepared CDs are suitable for optoelectronic and biological application. Additionally, the value of absolute quantum yield (QY) was measured to be 4.34%, which was comparable to the reported carbon dots.

Cytotoxicity and cells imaging. CCK-8 assay was used to evaluate the cytotoxicity of the CDs to living cells. As shown in Fig. 7, the cell viability were estimated to be greater than 80% after 24 h of incubation upon
addition of the as-prepared CDs over concentration ranged from 0 to 200 μg/mL, which confirmed the low toxicity, excellent biocompatibility and safety in vitro and in vivo applications of the as-prepared CDs\(^6,44\). Furthermore, in order to investigate the imaging capability, we induced the as-prepared CDs into onion root tip cells. The confocal laser scanning microscopy (CLSM) images of onion root tip cells labeled with the as-prepared CDs for 12 h at 25 °C were shown in Fig. 8. Obviously, the as-prepared CDs have penetrated into the cells and labeled both the cell nuclei and membrane and shown significant green fluorescence emission under the 405 nm wavelength excitation. This result indicated that the as-prepared CDs possessed good biocompatibility and could serve in bioimaging and drug delivery applications\(^{45,46}\).

**Conclusions**

In summary, the amino-functionalized CDs have been successfully prepared by a simple carbonization approach. This green, cheap and convenient process represented a potential advance for large-scale production. The as-prepared CDs possessed desirable amino function group on the surface and exhibited bright luminescence...
with absolute quantum yield (QY) of 4.34%, excitation-, pH-dependent and up-conversion fluorescence behaviors. Furthermore, the as-prepared CDs showed good stability, less toxicity, biocompatibility and excellent fluorescence imaging ability. In the future, the as-prepared CDs could be utilized promisingly in optoelectronic materials, photocatalysts, cellular imaging and drug delivery applications.

Methods

Materials. All chemicals, including chitosan (Sinopharm Chemical Reagent Co., Ltd. China), sulfuric acid (H₂SO₄; Beijing Chemical Works, China), sodium hydroxide (NaOH; Beijing Chemical Works, China) and phosphate buffer saline (PBS; Leagene, China), were in analytical grade and used without further purification. Deionized water was used throughout the whole experiment.

Preparation of CDs. CDs were prepared via carbonization of chitosan at 300 °C for 2 h at a heating rate of 5 °C/min under a nitrogen atmosphere. After cooled down to room temperature, the dark brown products were mechanically ground to fine powders. After that, obtained powders were dispersed in water, and the CDs were collected by filtrating for further characterization and use.

Characterization. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) micrographs were recorded on JEM-2100F (JEOL, Japan) operating at 200 kV. Atomic-force microscopy (AFM) investigation was done by a scanning probe microscope (BRUKER MULTIMADE 8, Bruker, USA). The crystal structure of samples was analyzed by an X-ray diffractometer (SHIMADZU XRD-6000, Shimadzu corporation, Japan) with Cu radiation (λ = 0.154 nm) at 40 kV and 40 mA. The Raman spectrum was recorded on Perkin Elmer Spectrum GX (Perkin Elmer, UK) at room temperature. Fourier-transform infrared (FT-IR) spectroscopy data were acquired using a Perkin Elmer Spotlight 400 imaging system (Perkin Elmer, UK). The X-ray photoelectron spectroscopy (XPS) measurements were performed on Thermo Scientific Escalab 250Xi XPS system (Thermo Fisher Scientific Ltd., UK) using Al Kα source. The temperature programmed desorption of CO₂ (CO₂-TPD) measurement was carried out on an AutoChem II 2920 analyser (Micrometrics, USA) fitted with a thermal conductivity detector (TCD). Ultraviolet-visible (UV-Vis) absorption spectra were carried out with UV2310II spectrometer (Techcomp, China). The fluorescence spectra were measured with a fluorescence spectrometer (F-7000, Hitachi High Tech CO., Japan). Fluorescence decay curves and photoluminescence (PL) absolute quantum yield (QY) for as-prepared CDs were measured at room temperature using an Edinburgh FLS 980 spectrometer (Edinburgh Instruments, UK) equipped with an integrating sphere under 310 nm excitation from a 450 W xenon lamp. The absolute fluorescence quantum yield was calculated as follows:

\[
\eta = \frac{E_B - E_A}{S_A - S_B}
\]

where η is the absolute fluorescence quantum yield, the indices “A” and “B” referred to the reference sample (deionized water) and carbon dots aqueous solution, separately, and S_A, S_B, E_A and E_B referred to the integral of the scans. The specific calculation was handled by using the Edinburgh FLS 980 software package (Quantum Yield Wizard)²⁷,⁴⁸.

Effect of pH on the PL of as-prepared CDs. The as-prepared CDs aqueous solutions were adjusted to the various target pH values by adding diluted H₂SO₄ or NaOH solution, then the corresponding fluorescence spectra were measured upon excitation at 310 nm separately.

Cytotoxicity evaluation. The cytotoxicity of as-prepared CDs was evaluated by a CCK-8 assay⁴⁴. Lewis Lung Carcinoma (LLC) cells were seeded at a density of 4 × 10⁵ cells per well in 180 mL culture medium and incubated for 24h. Then, the cells were treated with different concentrations of as-prepared CDs solution at 37 °C in a humidified incubator with 5% CO₂ for 24h. Then, 20mL of CCK-8 solution was added to each well and incubated

![Figure 8. CLSM images of onion root tip cells labeled with as-prepared CDs at 25 °C for 12 h (a) Bright field, (b) Fluorescent image excited with a 405 nm laser and (c) Overlap of the corresponding bright field image and the fluorescence image.](image-url)
for 1 h at 37 °C. The absorbance at 450 nm was measured using an infinite M200 microplate spectrophotometer (Tecan, Switzerland). The cell viability was expressed as percentage of absorbance relative to control, and the control was obtained in the absence of as-prepared CDs.

**Cell imaging.** Onion root tip cells were incubated with as-prepared CDs solution for 12 h at 25 °C, then washed with PBS for several times. The cellular localization was visualized using a confocal laser scanning microscope (SP-5, Leica, Germany) with laser excitation of 405 nm.49,50

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Author Contributions
X.Z. and X.L. conceived the project and designed the experiments; X.L. wrote the main manuscript text; X.L. and J.P. performed the experiments and analysed the data; X.Z. and F.X. supervised and directed the project; All authors reviewed the manuscript.

Additional Information
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