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High Quantum Yield Green-Emitting Carbon Dots for Fe(III) Detection, Biocompatible Fluorescent Ink and Cellular Imaging

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In the present work, we reported the luminescence of a green-emitting carbon dots (CDs) synthesized via solid state reaction method using diammonium hydrogen citrate and urea as a starting materials. The obtained green-emitting CDs shows strong absorption in the 350–450 nm region and gives intense green emission ($\lambda_{\text{max}} = 537$ nm) with quantum yield as high as 46.4% under 420 nm excitation. The obtained green-emitting CDs also demonstrates high photo-stability, which is evidenced by the fact that its emission intensity almost has no change under irradiation by a 365 nm UV lamp for 2 hours. Moreover, the obtained green-emitting CDs shows high sensitivity and selectivity for the detection of Fe^{3+} , and their emission intensity response towards Fe^{3+} ions is highly linear ($R^2 = 0.995$) over the concentration range from 25 to 300 μM , which could provide an effective platform for detection of Fe^{3+} . Mostly important, we further demonstrate that such photoluminescent green-emitting CDs exhibits low toxicity and are biocompatible for use with in cellular imaging. Combining with low cytotoxicity, good water solubility and excellent luminescence properties, green-emitting CDs could be used as a biocompatible fluorescent ink in future applications.

As a new comer of fluorescent nanoparticle, carbon dots (CDs) continue to fascinate a mankind because of their outstanding physiochemical properties such as high luminescence, low-toxicity, surface modification flexibility, super resistance to photobleaching, good biocompatibility and excellent water solubility^{1–4}. CDs is intensely recognized for their potential applications in bio-imaging, optoelectronics, photocatalysis, light-emitting diodes, sensing and energy conversion/storage devices^{5–11}. These excellent physiochemical properties and their numerous applications make CDs a promising alternative to organic dyes, polymer dots and semiconductors quantum dots that have been extensively studied over the past decades^{12,13}. However, the toxicity, poor water solubility and photobleaching, complex synthesis procedure and low quantum yield (QY) of the latter materials (organic dyes, polymer dots and semiconductors quantum dots) have limited their practical applications^{14–17}.

After a careful reviewing of the existing literatures, it is found that many different types of materials and approaches have been used to synthesize CDs. The reported raw materials vary from laboratory-produced chemicals to natural products, and approaches include laser ablation, pyrolysis, electrochemical oxidation, hydro/solvo-thermal reactions, and microwave treatment *et al.*^{15,18–22}. However, most of the resultant carbon dots emit blue and blue-green, and few are found to emit in longer wavelengths^{10,23–26}. In addition, the methods mentioned above are not able to produce large quantity of product. Moreover, carbon dots suffer from the difficulty in producing high quantum yield (QY) and long wavelength (>500 nm) emissive products, which are considered as a major disadvantage of CDs. Therefore, it is urgent to develop a new way to synthesize carbon dots that can emit a longer emission beyond blue region, so as to enrich the luminescence properties and diversify its applications.

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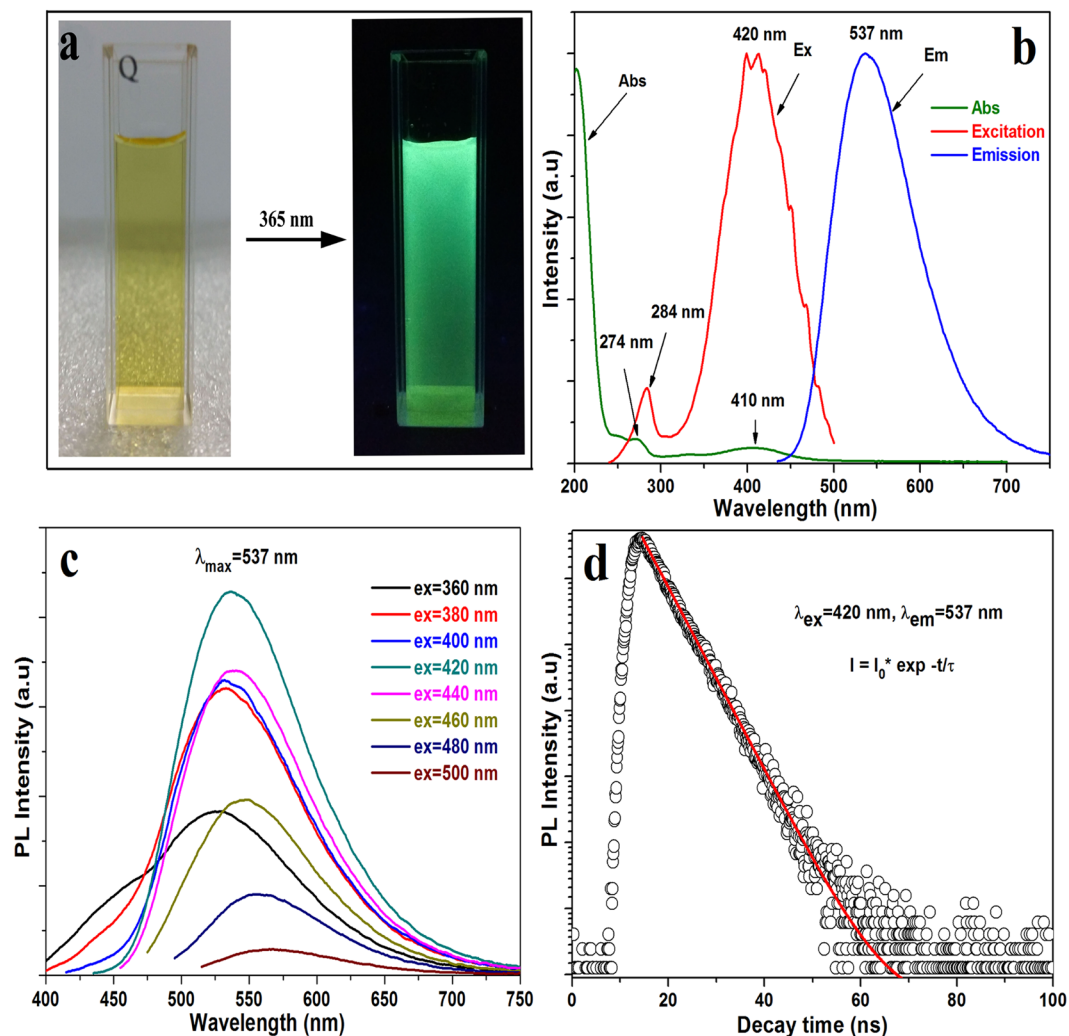


Figure 1. (a) Photos of the green-emitting CDs under sunlight (left) and UV irradiation (right). (b) UV-Vis absorption, excitation and emission spectra of the obtained green-emitting CDs. (c) The emission spectra of green-emitting CDs under different excitations. (d) Decay curve of the prepared green-emitting CDs under 420 nm excitation.

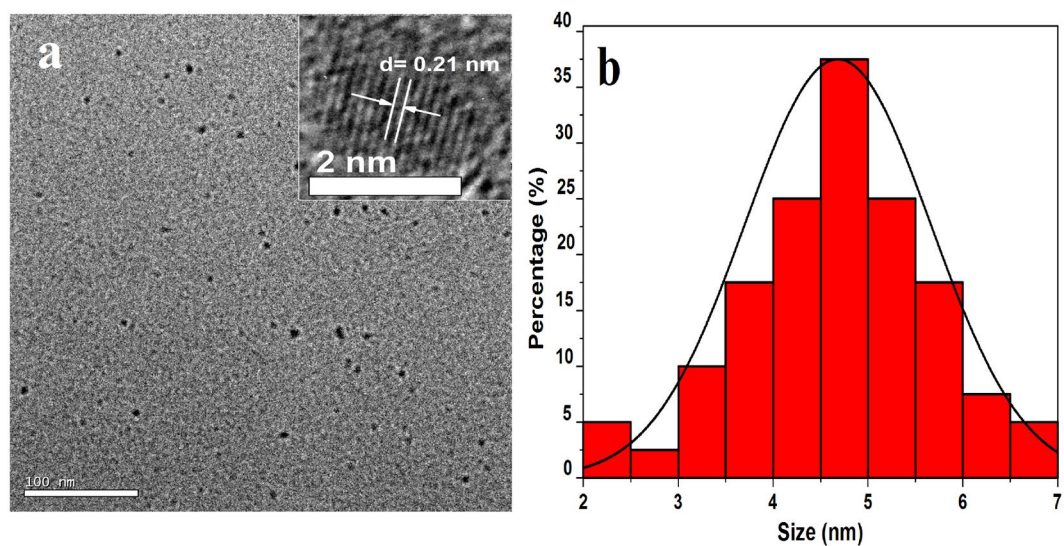


Figure 2. (a) The TEM and (inset) HRTEM image (b) and size distribution of the green-emitting CDs.

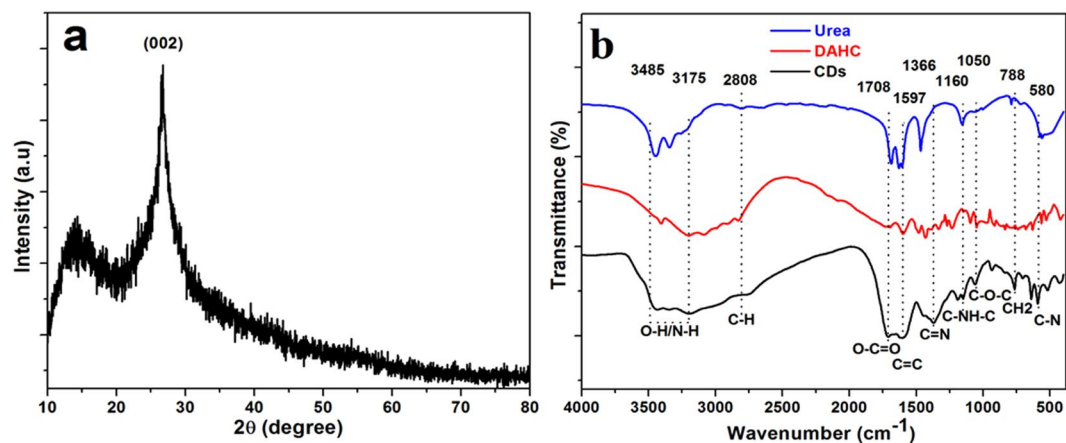


Figure 3. (a) The XRD profile of the prepared green-emitting CDs. (b) FT-IR spectrum of Urea, diammonium hydrogen citrate (DAHC) and obtained green-emitting CDs.

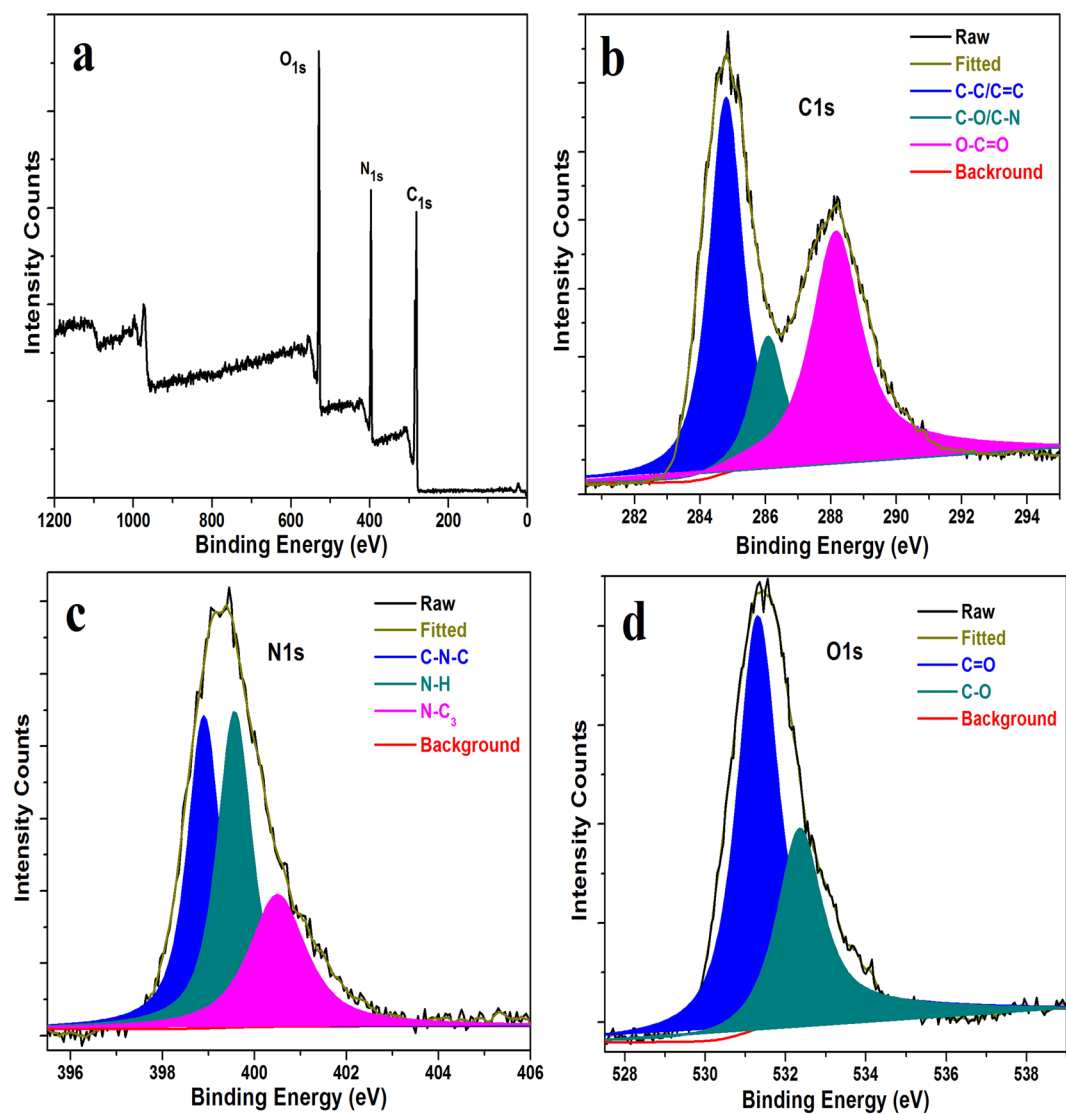


Figure 4. (a) Full XPS spectra of the obtained CDs. (b–d) High-resolution XPS spectra of the C 1s, N 1s and O1s of the obtained green-emitting CDs, respectively.

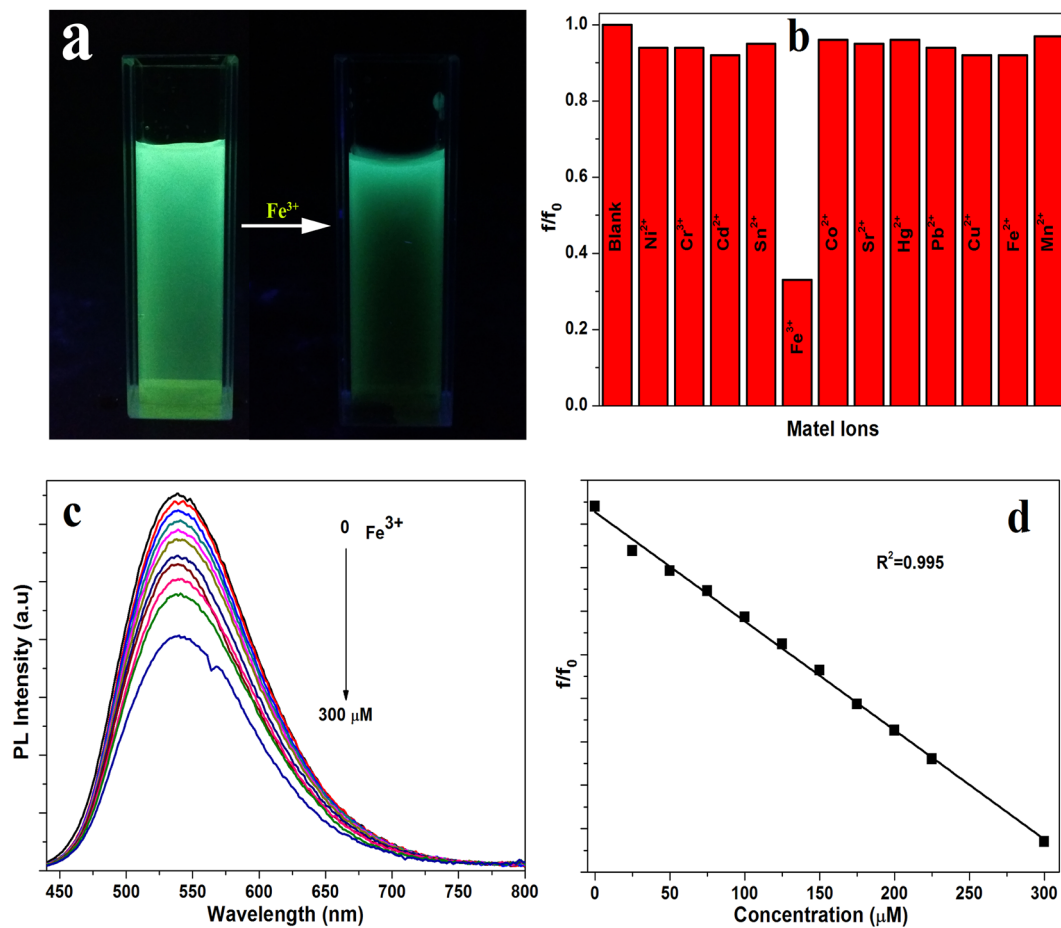


Figure 5. (a) photographs of the green-emitting CDs without and with Fe³⁺ ions under 365 nm UV lamp irradiation. (b) Emission intensity of the green-emitting CDs after adding different metal ions with same concentration. (c) The photoluminescence spectra of green-emitting CDs with different concentration of Fe³⁺ from 25 to 300 μM . (d) The dependence of f/f_0 on the concentration of Fe³⁺.

Herein, we report for the first time highly fluorescent green-emitting carbon dots through a facile solid state reaction method with quantum yield as high as 46.4% under 420 nm excitation. The resulting green-emitting CDs can emit bright and stable green luminescence at high salt concentration and show good photostability under continuous 365 nm UV lamp irradiation for 2 hours. The obtained green-emitting CDs possess excitation-dependent emission and short monoexponential fluorescent lifetime. Besides, the green-emitting CDs is shown to be able to detect Fe³⁺ ions with good sensitivity and selectivity over the concentration range from 25 to 300 μM . Because of their outstanding photoluminescence properties and low-cytotoxicity, green-emitting CDs is particularly suitable for biocompatible fluorescent ink and also fluorescent probe for cellular imaging. For their easy preparation and unique optical properties make these CDs promising for numerous applications in modern science and technology.

Results and Discussion

Photoluminescent properties of the green-emitting CDs. Different combinations of materials with diammonium hydrogen citrate were used to synthesize carbon dots, however, only the carbon dots synthesized by using diammonium hydrogen citrate with urea were found give green emission, and all the rest give blue emission (see supporting information Figure S1). As a result, urea with diammonium hydrogen citrate are ultimately selected due to their products yielded green emission with high quantum yield, indicating that green emissive products with highly quantum yield are dominate to synthesize carbon dots.

The obtained carbon dots are yellow under day light irradiation but green under a 365 nm UV lamp irradiation, which can be seen in Fig. 1a. The absorption and photoluminescence (PL) properties of the aqueous green-emitting CDs is characterized (Fig. 1b) by the ultraviolet-visible (UV-Vis) and PL spectrometer. In the UV-Vis spectrum, it can be seen clearly that there are two absorption peaks appeared at 274 and 410 nm. The absorption peak at 274 nm is attributed to carbonic core center and the peak catered at 410 nm is ascribed to surface or molecule center²⁷. Under 420 nm excitation, the carbon dots yield green emission at 537 nm (see Fig. 1b). The excitation spectrum obtained by monitoring the green emission at 537 nm is consistent with the result presented in the absorption spectrum. This behavior also indicates that there are at least two types of excitation energy trapping on the surface of CDs¹⁹. In addition, it is found that the green-emitting CDs exhibit excitation-dependent

Synthetic method	Precursor	Linear range	Emission range/QYs %	Limit of detection (LOD)	Reference
Hydrothermal method	alginate acid and ethanediamine	0.0–0.05 mM	Blue/0.7	10.98 μ M	39
	cellulose fibers	25–250 μ M	Blue/32.2	0.96 μ M	40
	L-glutamate	0–50 μ M	Blue/17.8	4.67 μ M	41
	potatoes	1.0–5.0 μ M and 5.0–50.0 μ M	Blue/15	0.025 μ M	42
	α -lipoic acid, sodium hydroxide and ethylenediamine	0–500 μ M	Blue/54.4	4 μ M	7
	dopamine and ethanediamine	50–300 μ M	Blue-green/9.4	10.8 μ M	43
Electrochemical synthesis	graphite rods	0–1 μ M	Blue/NA	2 nm	44
	ethanol	1–80 μ M and 80–800 μ M	Blue/10.04	0.04 μ M	45
	Graphite electrode	10–200 μ M	Blue/11.2	1.8 μ M	46
Hummers method	GO nanosheets	14.32–143.2 μ M	Blue/0.7	17.9 μ M	47
Microwave irradiation	Chitosan, glacial acetic acid and EDA	0.179–32.22	Blue/20.1	0.179 μ M	48
Solid-state reaction	Diammonium hydrogen citrate and urea	0–300 μ M	Green/46.4	19 μ M	This work

Table 1. Analytical performances of recent reported sensors for the detection of Fe^{3+} .

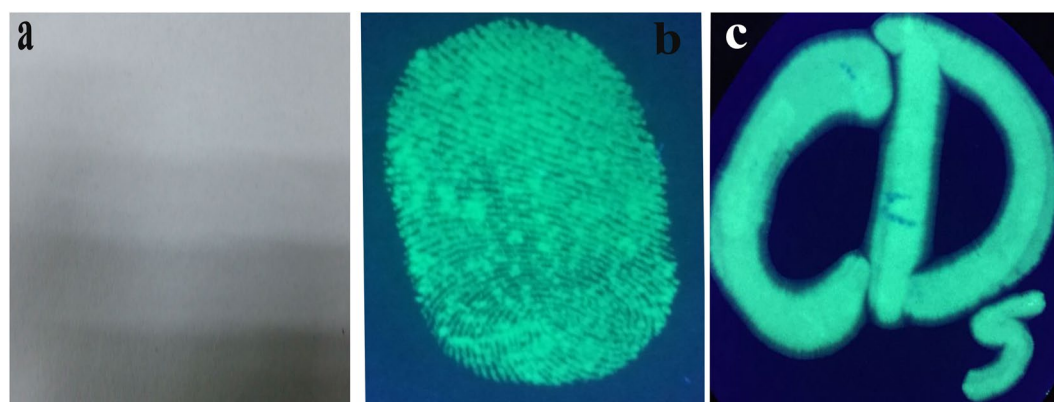


Figure 6. (a) Green-emitting CDs formed fingerprint under daylight (b) and 365 nm UV lamp irradiation. (c) Fluorescent characters on filter paper under UV excitation.

emission as shown in Fig. 1c. When the excitation wavelength is increased from 360 to 500 nm with the increment of 20 nm, the emission peak is red shifted from 525 to 570 nm. The excitation-dependent emission behavior of green-emitting CDs is related to be the non-uniform particle size and distribution of different surface states^{7,28,29}. TEM image confirms that the size of the particle is not very uniform (see Fig. 2). The QYs of the green-emitting CDs is determined to be respectable in either organic solvent (e.g. ethanol, 46.4%, Figure S2) or DI water (15.5%, Figure S3) under 420 nm excitation by using a rhodamine 6 G (95%) solution as a reference. To best of our knowledge, this is the highest QY value for the green-emitting CDs reported so far. Figure 1d presents the decay curve of the obtained green-emitting CDs with excitation and emission wavelengths in 420 and 537 nm respectively. The decay curve can be fitted by a single exponential function:

$$N(t) = A_0 \exp(-t/\tau) \quad (1)$$

The decay lifetime was determined to be 7.2 ns, and such a short lifetime indicates that the luminescence mechanism of our obtained green-emitting CDs is the radiative recombination nature¹⁹.

High stability is one of the important factors in nano-size fluorescent materials, which plays a significant role to demonstrate their application in various fields. The photo-stability and salt (KCl) effect on the green-emitting CDs have been investigated. It is confirmed that green-emitting CDs is highly stable under continuous irradiation of a 365 nm UV lamp for two hours (supporting information Fig. S4a). Furthermore, the prepared green-emitting CDs has favorable ionic strength at high salt (KCl) concentration of about 2 M (supporting information Fig. S4b). Moreover, the green-emitting CDs is confirmed to be relatively stable over the pH range from 2 to 9.5 as shown in the (supporting information Fig. S5).

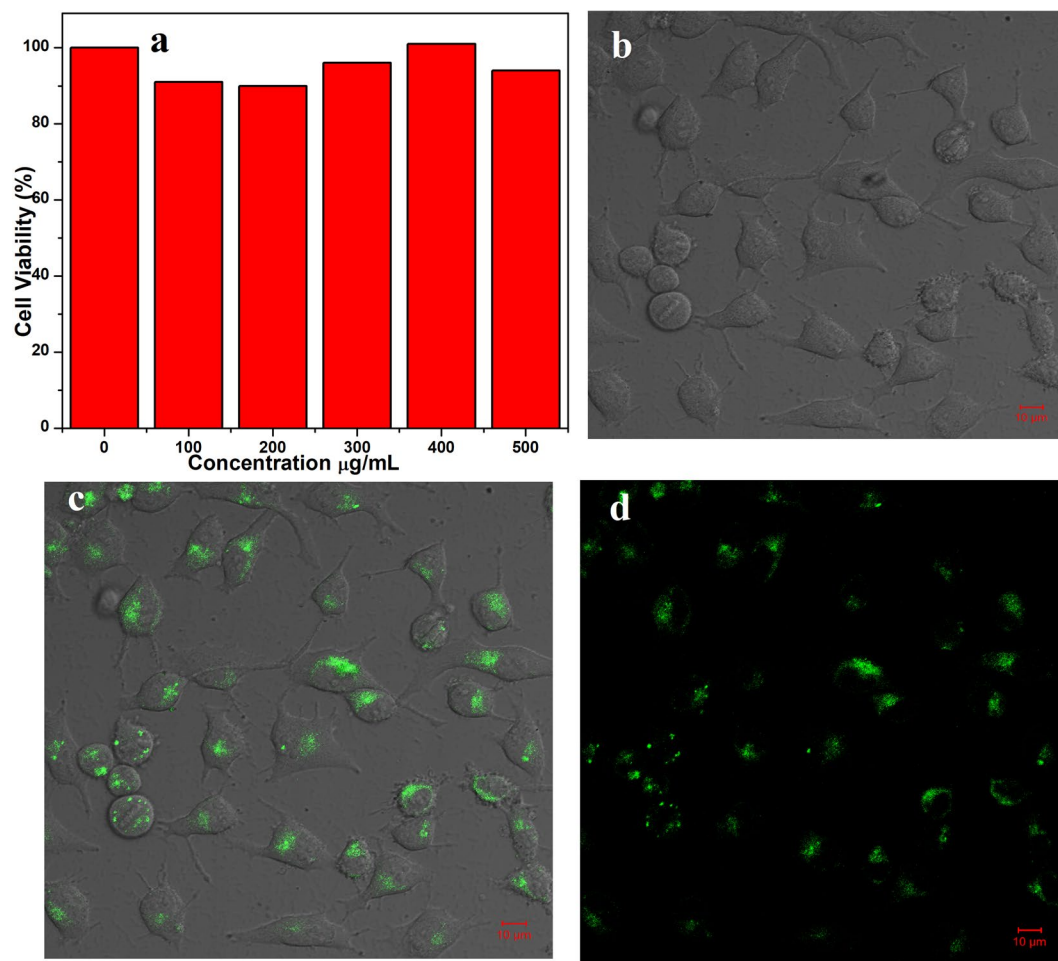


Figure 7. (a) Cellular toxicity assessment of green-emitting CDs. (b) Bright field image (c and d) Confocal fluorescent images of the green-emitting CDs under 405 nm laser excitation.

The obtained green-emitting CDs in aqueous state exhibits a long-term homogeneous phase without any noticeable precipitation at room temperature. The TEM images and size distribution of the prepared green-emitting CDs are displayed in Fig. 2a, from which it can be seen clearly that the obtained particles are scattered in regular manner and possess closely spherical pattern. The inset HRTEM image demonstrate the crystallinity of the green-emitting CDs with a lattice parameter of 0.21 nm, which agrees with (100) lattice fringes of graphite³⁰. The size of the obtained green-emitting CDs is not very uniform and it is distributed in the ranges from 2 to 7 nm. The average size of the green-emitting CDs is 4.6 nm as displayed in Fig. 2b.

The XRD profile of the green-emitting CDs shows (002) peak centred at 26° as shown in the Fig. 3a. The result is similar to that of the graphite, showing highly disordered carbon atoms^{31,32}. The structure and composition of green-emitting CDs were characterized by FT-IR spectroscopy. As shown in Fig. 3b, the synthesized green-emitting CDs show main absorption bands related to OH/NH stretching vibration at 3435 to 3175 cm^{-1} . These functional groups improve the hydrophilicity and stability of green-emitting CDs in aqueous systems^{3,33}. The weak peak at 2808 cm^{-1} is related to the C-H band stretching vibration. The peaks at 1708 and 1597 cm^{-1} are ascribed to the O-C=O and C=C stretching vibrations, respectively²³. Moreover, the CH/CN band is stretching at 1366 cm^{-1} on the surface of the green-emitting CDs. The characteristic stretch band of the amine H-N bond is at 1150 cm^{-1} . The IR bands appear within the range of 1050 to 580 cm^{-1} , which correspond to the stretching vibration of C-O, CH₂ and C-N, respectively³⁴. The unsaturated carbon bonds were further examined by the Raman spectrum, which shows the feature of D band at 1375 cm^{-1} and G band at 1596 cm^{-1} (supporting information, Fig. S6). The ratio of the relative intensity of D band and G band (I_D/I_G) is about 0.96, indicating the disorder nature of the green-emitting CDs with poor crystalline framework³².

X-ray photoelectron spectroscopy (XPS) measurements were performed for the surface elemental analysis of green-emitting CDs. The wide XPS spectrum of the green-CDs as shown in Fig. 4a clearly reveals that carbon (284.6 eV), nitrogen (398.8 eV), and oxygen (531.3 eV) are presented at the surface of green-emitting CDs. In the expanded XPS spectra (Fig. 4b), the C1s band can be deconvoluted into three peaks, which were assigned to sp² carbons (C=C, 284.5 eV), sp³ (C-O/C-N, 286.1 eV), carboxyl carbons (O-C=O, 288.1 eV). The N1s band can be deconvoluted into three peaks (Fig. 4c), which were corresponded to (C-N-C, 398.8 eV), amino

N (N-H, 399.5 eV) and (C₃-N, 400.5 eV). The oxygen containing groups are also presented in the surface of the green-emitting CDs (Fig. 4d), the O1s band contains two peaks associated to sp² (C=O, 531.3 eV) and sp³ (C-O, 532.3 eV)^{35,36}.

Application of green-emitting CDs

Detection of Fe³⁺ in an aqueous solution. The Fe³⁺ plays an important role in life due to their essential function in oxygen metabolism, oxygen transport, transfer of electrons and catalytic procedures³⁷. Recently, carbon fluorescent dots have been used as a fluorescent probe for heavy metal ions, showing good sensitivity and selectivity towards different metal ions. The photographs in Fig. 5a show the emission of green-emitting CDs before and after adding Fe³⁺ ions. Many common metal ions have been used for detection (Fig. 5b), but only Fe³⁺ is able to quench the fluorescence of green-emitting CDs, suggesting the green-emitting CDs have a potential to detect Fe³⁺ ions via luminescence measurements. The observed fluorescent quenching by Fe³⁺ ions could be attributed to its strong interaction with surface groups of green-emitting CDs, which facilitate the transformation of photoelectron from CDs to Fe³⁺ ions^{7,38}. Figure 5c shows the fluorescent spectra of the green-emitting CDs aqueous solution containing different concentration of Fe³⁺ ions: 25, 50, 75, 100, 125, 150, 175, 200, 225 and 300 μM. The photoluminescence spectra are decreasing by regular manner adding the different concentration of Fe³⁺ (excitation wavelength 420 nm). The ratios between (f/f_0) and Fe³⁺ concentration have a good linear relationship with correlation coefficient R² of 0.995 as shown in Fig. 5d. The limit of detection (LOD) is calculated to be 19 μM using the 3 times σ/K ratio. The observed results clearly illustrating that the green-emissive CDs have a good sensitivity and selectivity towards Fe³⁺ ions, which can be used as a practical application. The analytical performances of recent reported sensors for the detection of Fe³⁺ are summarized in (Table 1).

Biocompatible fluorescent ink. The low/non-toxicity at high concentration of green-emitting CDs indicates that it can possibly be used as a new type of biocompatible fluorescent ink. Figure 6a and b, show the green-emitting CDs based fluorescent fingerprint on filter paper under sun light and UV lamp (365 nm) irradiation. Figure 6c shows the fluorescent characters on filter paper irradiated by 365 nm UV beam. The carbon dots based fluorescent ink doesn't contaminate fingers and can be easily washed off from water^{4,27}.

Cellular imaging in HeLa cells. Carbon dots have a promising candidate for biological applications because of their non/low toxicity, good stability and highly luminescent materials^{49–51}. The cytotoxicity of the green-emitting CDs was evaluated by using a standard MTT assay with HeLa cells⁸. As shown in the Fig. 7a, the average cell viability is above 90% with concentrations ranging from 100 to 500 μg/mL for 24 hours. The result suggests that the green-emitting CDs has very low cytotoxicity and good biocompatibility with high concentration about 500 μg/mL. Figure 7b shows the bright field image of the HeLa cells. After incubating the green-emitting CDs to HeLa cells for imaging, cells emitted a remarkable green fluorescence (Fig. 7c and d) and showed good morphology under confocal laser scanning microscopy. The fluorescence signals reveal that green-emitting CDs is mainly inside the cell membrane, suggesting that the green-emitting CDs can pass through cell membrane and enter into the cells cytoplasm. These results indicate good cell permeability of the green-emitting CDs towards HeLa cells, which further confirm the merits of the green-emitting CDs.

Conclusion

In this work, we report the optical properties and possible applications of a green-emitting CDs synthesized by solid state reaction approach. Under 420 nm excitation, the obtained CDs emits bright green emission with quantum yield of 46.4%, which is the highest QY value for the green-emitting CDs reported so far. Besides, the obtained green-emitting CDs is proved to have favorable ionic strength, high stability, excellent water solubility and excitation dependent emission. The synthesized carbon dots have been demonstrated the potential to be used as a sensor of Fe³⁺ ions, fluorescent based-ink and cell marker. The low cost, biocompatibility, and low-toxicity of the green-emitting CDs and their distinct PL properties indicate that green-emitting CDs could potentially be synthesized on an industrial scale and could be used for numerous possible applications.

Methods

Materials. Diammonium hydrogen citrate, urea, L-cystiene, HD-met-oH, glycine, L-glutathione (GSH) and the salts (PbCl₂, Cr(NO₃)₃, CuCl₂, HgCl₂, NiCl₂, FeCl₂, FeCl₃, CdCl₂, CoCl₂, SnCl₂, HgCl₂, MnCl₂ and SrCl₂) were purchased from Sinopharm Chemical Reagent Co., Ltd, Rhodamine 6 G (99%) was purchased from Adamas Reagent Co., Ltd. All the chemicals were of analytical grade and used without further purification. The deionized water was used throughout the experiments.

Synthesis of green-emitting CDs. For a typical synthesis process diammonium hydrogen citrate (0.2 g) and urea (0.2 g) were grinded homogeneously in agate mortar, and then the obtained mixture was heated in an oven at 180 °C for 1 hour in air. After the reaction was over, the resulting product was dissolved in water. The obtained solution was purified in a centrifuge at high speed (12000 rpm, 5 min) to remove the large particles. Afterword, the solution was further filtered via 0.22 μm filter membrane to obtain the pure CDs. The obtained solution of CDs was dispersed in deionize water for further characterizations.

Characterizations. The morphology and size of the samples was observed by (FEI Tecnai F30 transmission electron microscope (TEM) operating at 300 kV). Photoluminescence (PL) and photoluminescence excitation (PLE) spectra were recorded using a Fluorolog-3 Spectrofluorometer equipped with a 450 W Xenon light source. Ultraviolet-visible (UV-Vis) absorption spectra were recorded using a Lambda 950, Perkin-Elmer

(USA) UV-Vis-NIR spectrophotometer. The phase purity of fluorescent CDs was analyzed by X-ray diffraction (XRD) using a Bruker D2 PHASER X-ray Diffractometer with graphite monochromator using Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$), operating at 30 kV and 15 mA. Fourier transform infrared spectroscopy (FT-IR) was recorded a Nicolet 670 spectrophotometer at room temperature. X-ray photoelectron spectra (XPS) were acquired using a (XPS, PHI- 5702, Physical Electronics) with a monochromatic Al K α irradiation source. The PL decay curves were measured by a FLS-920T fluorescence spectrophotometer with an nF900 nanosecond flash lamp as the light source. Raman spectroscopy was measured using a (JY-HR800 micro-Raman spectroscopy) with a 532 nm wavelength of YAG laser.

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Author Contributions

W.K. and D.W. conducted the most of investigation for the samples. W.K. wrote the main paper. D.W. and W.Z. designed the concept and the experiment method of the research. D.W. and Y.W. supervised the project, had given valuable advices on the proceeding of this work and revised the manuscript. Z.T. and S.D. supported the characterization of the samples. D.X. and X.M. revised the manuscript. All authors discussed the results and commented on the manuscript at all stages.

Additional Information

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