Supporting information

Intrinsically fluorescent carbon dots with tunable emission derived from hydrothermal treating glucose in the presence of monopotassium phosphate

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Chemicals
D-(+)-glucose (99.5%, Sigma), KH2PO4 (99.5%, Merck) and Ethanol (99.9%, Merck).

Characterizations
Ultraviolet-visible (UV-Vis) and photoluminescence (PL) spectra of the carbon dots (C-dots) solution were measured by Shimadzu UV 3101 PC and Perkin Elmer LS55 Fluorescence Spectrometer at room temperature, respectively. Quartz cells with 10 mm path length were used for all spectra testing. Life time of C-dots was measured by the 405 nm line of a picosecond pulsed laser diode head (PicoQuant PDL 800-B). For the life time testing, the PL of the as-synthesized C-dots was measured using a monochromator (Acton SpectroPro 2300i) and detected with a liquid N2 cooled CCD camera (Princeton, Spec-10:100). Then the lifetime results were recorded with Time-correlated Single Photon Counting (TCSPC) Module and Picosecond Event Timer (PicoHarp 300). The particle morphology of C-dots was studied by using transmission electron microscope (TEM) at an accelerating voltage of 300 kV (TEM, CM 300 FEG-Philips). Dry C-dots powder was obtained by freeze drying followed by vacuum drying at 60 °C. The carbon structure of the C-dots was determined by RENISHAW Raman Microscope with 514 nm laser radiation source. Their chemical structure was examined by using Fourier Transform Infrared (FTIR) spectroscopic (Perkin Elmer FT-IR 2000), where the FTIR sample was prepared by pressing the mixture of C-dots and potassium bromide (KBr) into a thin pellet. The surface structure of C-dots was investigated using X-ray photoelectron spectroscopy (XPS) (ESCALAB 200i-XL, Thermo Scientific). The confocal images of HepG2 cells cultured in the presence of C-Green were recorded by using Olympus Fluoview 1000 confocal laser scanning microscope.

Cell culture
HepG2 cells (American Type Culture Collection) were used for the cell study. The cell line was cultured in Dulbecco’s Modified Eagle Medium [high Glucose (4.5g/L), with Sodium Pyruvate and L-glutamin] (PAA laboratories cell Culture Products, Austria), containing 10% Fatal Bovine Serum (Invitrogen, USA), 1% antibiotic with 100 UI/ml Penicillin and 100ug/ml Streptomycin (Invitrogen, USA). The incubation condition was set at 37°C in a humidified atmosphere of 95% air and 5% CO2. The culture medium was changed 2-3 times a week and cells were passaged serially using trypsin/EDTA (Invitrogen, USA).

For the cell viability study, the HepG2 cells cultured in the presence of C-Green was studied by MTS
5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The HepG2 cells were first grown by using a 96 well plates (4,000 cells/well) and then cultured in culture media with various concentration of C-Green (0, 0.05, 0.125, 0.25, 0.5 and 0.625 mg/mL) at 37°C in 5% CO₂. After culturing for 72 hours, MTS/phenazine methosulfate solution was used to replace the growth medium and then the HepG2 cells were incubated for another 3 h. The Benchmark Plus microplate spectrophotometer (Bio-RAD) was used to measure the optical absorbance of each cell growth well (λ = 490 nm). The cell viability at each C-Green concentration were averaged from three repeated testing.

For confocal imaging HepG2 cells, the cells (15,000 cells/well) were seeded in an 8 well chamber (LAB-TEK, Chambered Coverglass System). The culture medium and the growth environment is the same as that for cell viability test. After 24 hours, fresh DMEM containing 0.5 mg/mL C-Green was used to replace the culture medium.

**Determination of quantum yield**

The quantum yield (Φ) of the as-synthesized C-dots was determined by comparing the integrated PL and absorbance intensities with the reference fluorophore. Quinine sulfate (Φ=0.54⁴) is used as the reference fluorophore for determination of the quantum yield of C-Blue. Fluorescein (Φ=0.79⁷) is used as the reference fluorophore for determination of quantum yield of C-Green. As a standard measurement, the quinine sulfate was dissolved in 0.1 M H₂SO₄ (refractive index (η) =1.33) and the fluorescein was dissolved 0.1 M NaOH (η =1.33). C-dots were dispersed in deionized water (η =1.33). All samples were measured by using UV-Vis spectrometer to obtain the absorbance intensities. In addition, when conducting UV-Vis measurement by using the 10 mm fluorescence cuvette, the absorbance values are kept below 0.1 in order to minimize re-absorption effects. The samples were then measured by PL spectrometer in order to get the PL emission intensity at the excitation wavelength with which the C-dots and the reference have the same UV absorbance. Then the quantum yield was calculated by using the following equation:

$$\phi = \frac{\int \frac{I}{A} dA}{\int \frac{I}{A} dA} \times \frac{\eta}{\eta} \times \frac{\eta}{\eta}$$

Where Φ is the quantum yield, I is the integrated PL intensity, η is the refractive index, and A is the absorbance intensity. The subscript R refers to the reference fluorophore of known quantum yield.

**Table S1. Quantum yield of C-Blue**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Integrated PL intensity (I)</th>
<th>Absorbance intensity at 350 nm (A)</th>
<th>Refractive index (η)</th>
<th>Quantum yield (Ô)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine sulfate</td>
<td>16363.6</td>
<td>0.0313</td>
<td>1.33</td>
<td>0.54⁴</td>
</tr>
<tr>
<td>C-Blue</td>
<td>721.8</td>
<td>0.0313</td>
<td>1.33</td>
<td>0.024</td>
</tr>
</tbody>
</table>


**Table S2. Quantum yield of C-Green**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Integrated PL intensity (I)</th>
<th>Absorbance intensity at 440 nm (A)</th>
<th>Refractive index (η)</th>
<th>Quantum yield (Ô)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescein</td>
<td>3507.4</td>
<td>0.0052</td>
<td>1.33</td>
<td>0.79⁷</td>
</tr>
</tbody>
</table>
C-Green  
| 50.9 | 0.0052 | 1.33 | 0.011 |


**Fig. S1:**

Fig. S1. Digital photos of the achieved supernatant derived from hydrothermal treating glucose in the presence of KH$_2$PO$_4$ (a) and without KH$_2$PO$_4$ (b).

**Fig. S2:**

Fig. S2. TEM images of carbon aggregates synthesized through hydrothermal treatment of glucose (1 mg/ml) at 200 °C for 12 hours without KH$_2$PO$_4$.

**Fig. S3:**

![Graph](image)**(a)**

\[ I(t) = I_0 \exp \left(-\frac{t}{\tau}\right) \]

\[ I_0 = 2.05815 \]

\[ \tau = 2.00716 \text{ ns} \]

![Graph](image)**(b)**

\[ I(t) = I_0 \exp \left(-\frac{t}{\tau}\right) \]

\[ I_0 = 2.96562 \]

\[ \tau = 1.76933 \text{ ns} \]
Fig. S3. Lifetime determination of C-Blue (a) and C-Green (b).

Fig. S4:

Fig. S4. Dependence of PL emission intensity against excitation time for C-Blue (a) and C-Green (b) in DI water. The C-blue is continuously excited at 350 nm by Xe lamp while the C-Green is continuously excited at 440 nm by Xe lamp.

Fig. S5:

Fig. S5. Normalized PL emission spectra of C-Blue at 350 nm excitation (a) and C-Green at 440 nm excitation (b) in aqueous solution at different pH value show there is no shift with PL emission at the tested pH range. Dependence of the PL emission intensity against pH value for C-Blue (inset in (a)) and C-Green (inset in (b)) show the C-dots are quite stable in the tested pH range.
Fig. S6: Raman spectra of C-Blue (a) and C-Green (b), respectively.

Fig. S7: C1s XPS spectra of C-Blue (a) and C-Green (b), respectively. Since the size of C-blue is smaller than that of C-Green, the relative intensity of surface functional groups such as C=O and C=C in C-Blue are higher than those in C-Green.

Fig. S8: Z-axis scanning confocal images of HepG2 cells cultured in the presence of C-Green (0.5
mg/ml) for 24 hours ($\lambda = 488$ nm).

**Fig. S9:**

![Cell viability of HepG2 cells cultured at different concentrations of C-Green and tested by MTS assay.](image)

**Fig. S9** Cell viability of HepG2 cells cultured at different concentrations of C-Green and tested by MTS assay.