Electronic Supplementary Material (ESI) for Analytical Methods.

Separation of Carbon Quantum Dots on a C18 Column by a binary gradient elution via HPLC

Supplementary Data

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Overview The measurements of Quantum yield (QY) of Carbon Quantum Dots (CQDs) were provided. A comparison of separating CQDs samples with mobile phase of water and methanol or acetonitrile/water and acetonitrile/methanol was displayed. Chromatographic behaviors of CQDs under different temperature, different excitation wavelength and different emission wavelength were showed. The resolution of fraction 14 and 15 under different temperature and under different flow rate were calculated. The detail of characterization of CQDs through X-ray photoelectron spectroscopy (XPS) was provided. Repeatability tests of CQDs under the optimized chromatographic conditions were accomplished.

EXPERIMENTAL SECTION

1. Measurement of the Quantum Yield.

The QYs of the unseparated CQDs and the collected fractions were determined by comparing the integrated photoluminescence intensities and the absorbances of the samples with the reference quinine sulfate.[1-3] Quinine sulfate was dissolved in 0.1 M H_2SO_4 (QY of 0.577 at 350 nm[1]), then diluted with 0.1 M H_2SO_4 into different concentrations as standards to calculate the QYs of the unseparated CQDs and the collected fractions.[4] The CQDs samples were dissolved in ethanol and diluted into different concentrations of solution. The absorbances of all the sample solutions at 320 nm were recorded with UV-Vis spectrophotometer. Photoluminescence emission spectra of all the sample solutions were measured by HITACHI F-7000 fluorescence spectrometer at an excitation wavelength of 320 nm. In order to avoid inner filter effects minimize, absorbance of the sample solutions were kept under 0.05 at the excitation wavelength of 320 nm.[3] The refractive index of 0.1 M H_2SO_4, the deionized H_2O and ethanol were 1.33, 1.33 and 1.36 respectively.[5] The QY was calculated according to the following equation:

$$Q = Q_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2}$$

Q was the QY, I was the measured integrated emission intensity, η was the refractive index, and A was the absorbance. The subscript R denoted values for the reference standard. An excitation slit width of 5.0 nm and an emission slit width of 5.0 nm were used to excite the photoluminescent CQDs samples. The excitation wavelength was 320 nm. The QYs of the seventeen fractions were in Table S-3 and the QY of the unseparated CQDs was 0.42.

2. The XPS analyses.

The solution of the fractions (a total of 17 fractions) were dropped onto a cover glass (pre-cleaning, 2×2 cm) respectively, vacuum dried. A spot of CQDs was formed to be analyzed by XPS. The XPS spectrum showed that there were six functional groups in these fractions, such as sp^2C (C=C), sp^3C (C-C), C-OH, C-O-C, C=O, –COO (Fig. S-6). In Table S-3, it showed that these fractions of CQDs had different QYs and different number of functional groups.

FIGURES

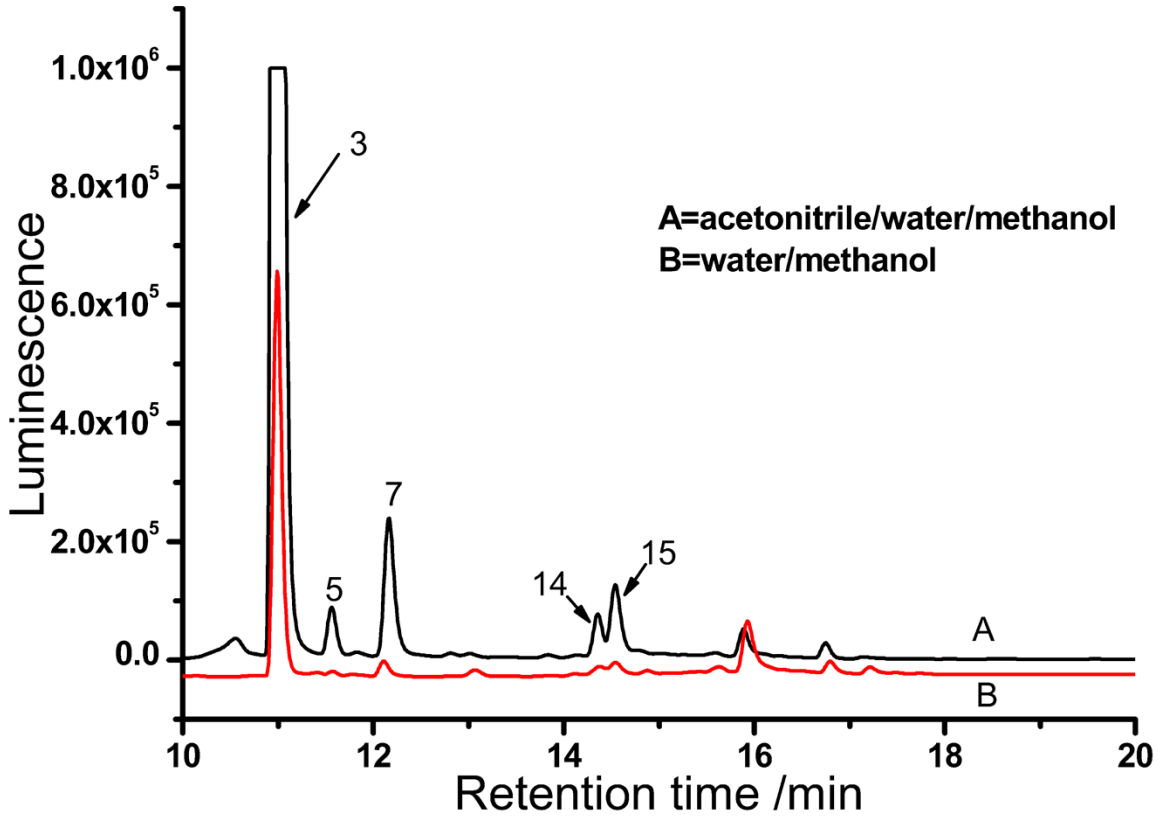

Fig. S-1. A comparison of separating CQDs samples with mobile phase of water/methanol or acetonitrile/water - acetonitrile/methanol. The flow rate was 1.4 mL min^{-1}. The temperature was 25 °C. The excitation and emission wavelengths were 320 and 460 nm, respectively.The injection volume of the CQDs samples was 1 μL (concentration, 0.05 mg mL^{-1}). When water/acetonitrile and acetonitrile/methanol were used as mobile phase, the separation was finished under the optimized chromatographic conditions (in chromatography *A*). When solvent A (water/methanol, 1/9, v/v) and B (pure methanol) were used as mobile phase, the optimized gradient elution program was as follows: 0 to 5 min with 100% solvent A; 5 to 8 min with solvent A from 100 to 45%, solvent B from 0 to 55%, then maintaining 2 min; 10 to 13 min with solvent A from 45% to 0, solvent B from 55 to 100%, then maintaining until 45 min (in chromatography *B*).

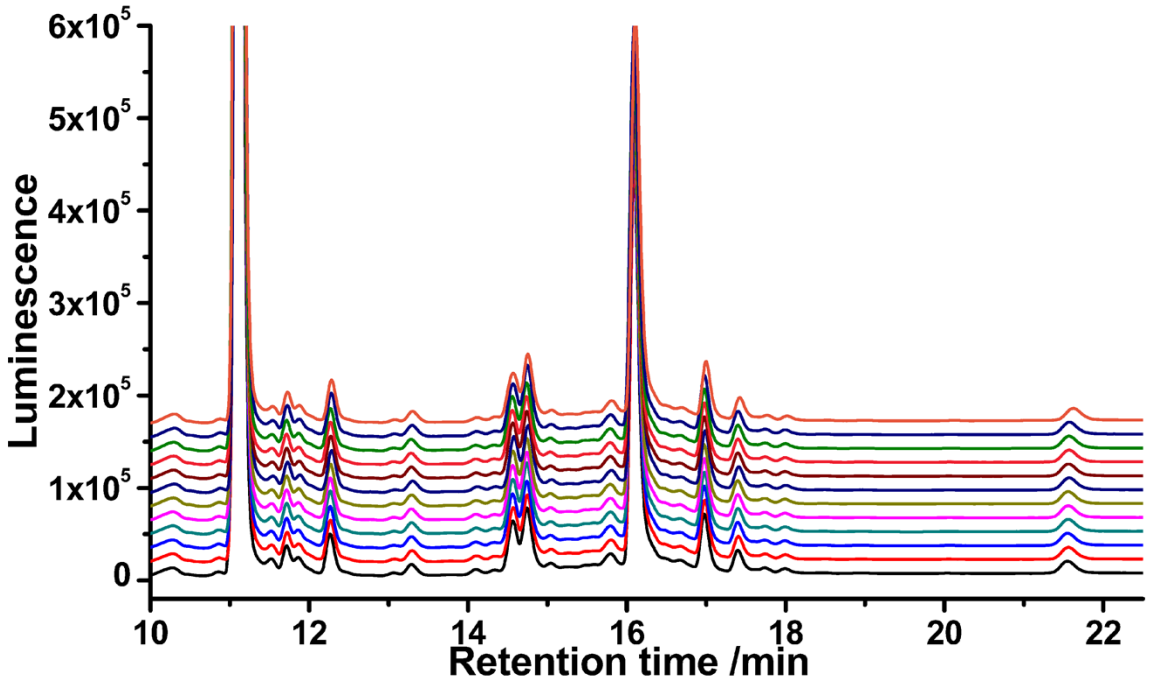

Fig. S-2. Repeatability tests of CQDs under the optimized chromatographic conditions. The injection volume of the CQDs samples was 0.5 μL (concentration, 0.5 mg mL^{-1}) and a total of injection were twelve runs.

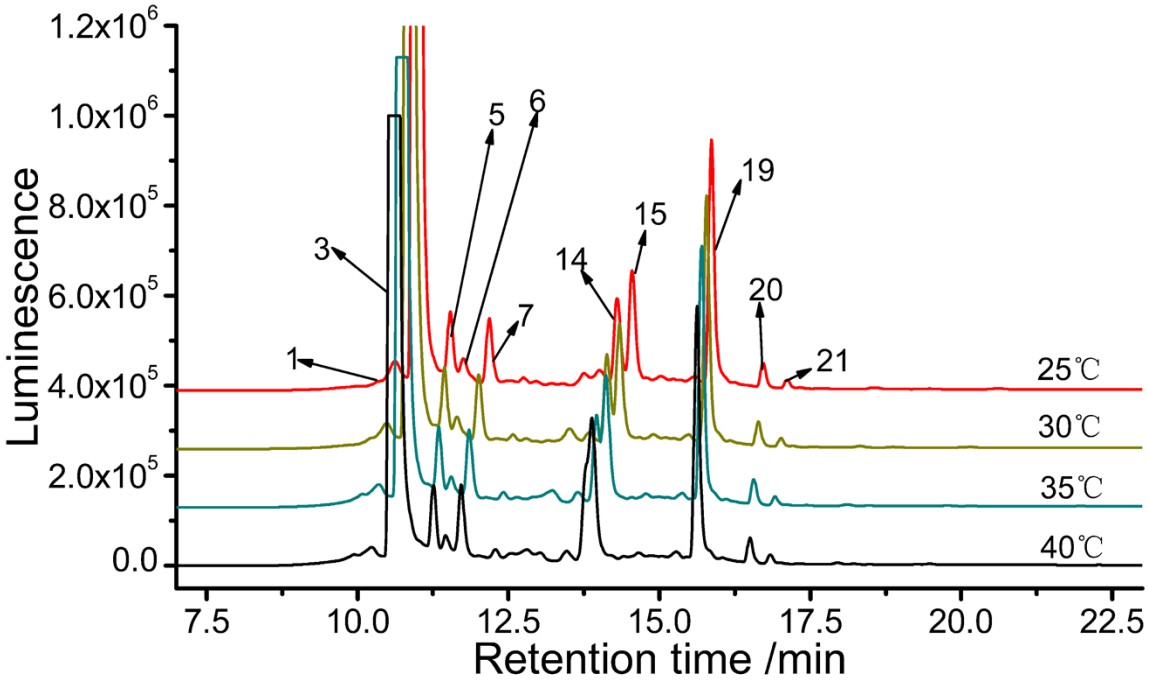

Fig. S-3. Chromatographic behavior of CQDs under different temperature. Flow velocity was 1.4 mL min^{-1}. The excitation wavelength was 320 nm and the emission wavelength was 460 nm. The injection volume of the CQDs samples was 0.5 μL (concentration, 0.5 mg mL^{-1}). The temperature was from 25 to 40 ℃ with an interval of 5 ℃.

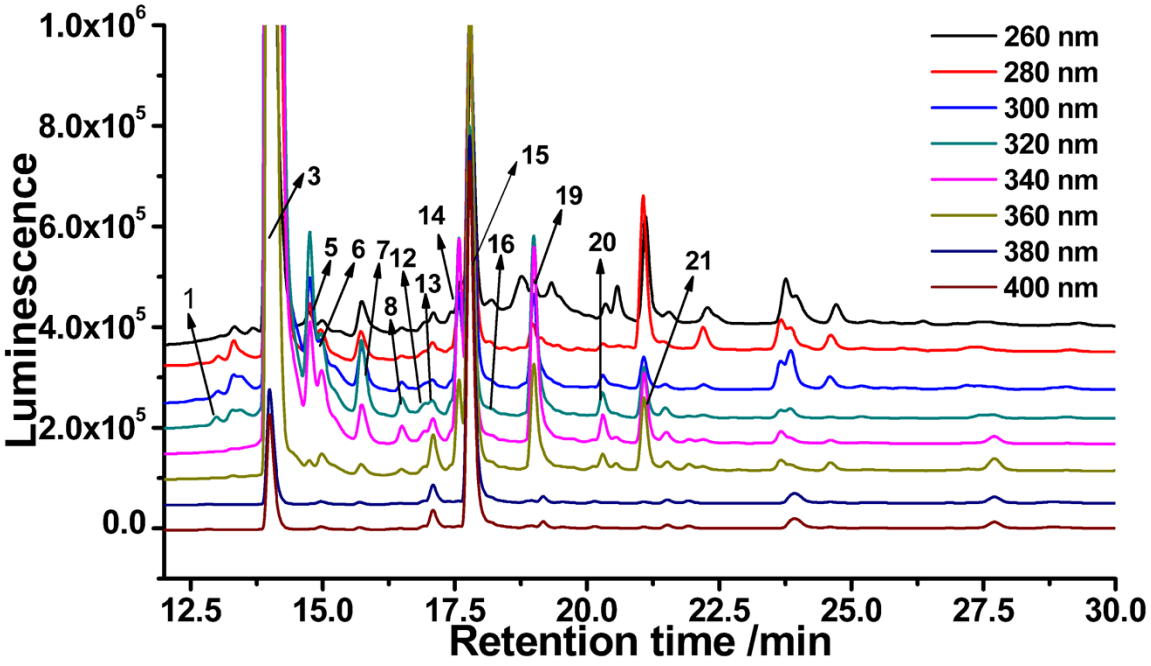

Fig. S-4. Chromatographic behavior of CQDs under different excitation wavelengths. Flow velocity was 0.8 mL min^{-1}. The temperature was 25 ℃ and the emission wavelength was 460 nm. The injection volume of the CQDs samples was 0.5 μL (concentration, 0.5 mg mL^{-1}). The excitation wavelengths were from 260 to 400 nm with an interval of 20 nm.

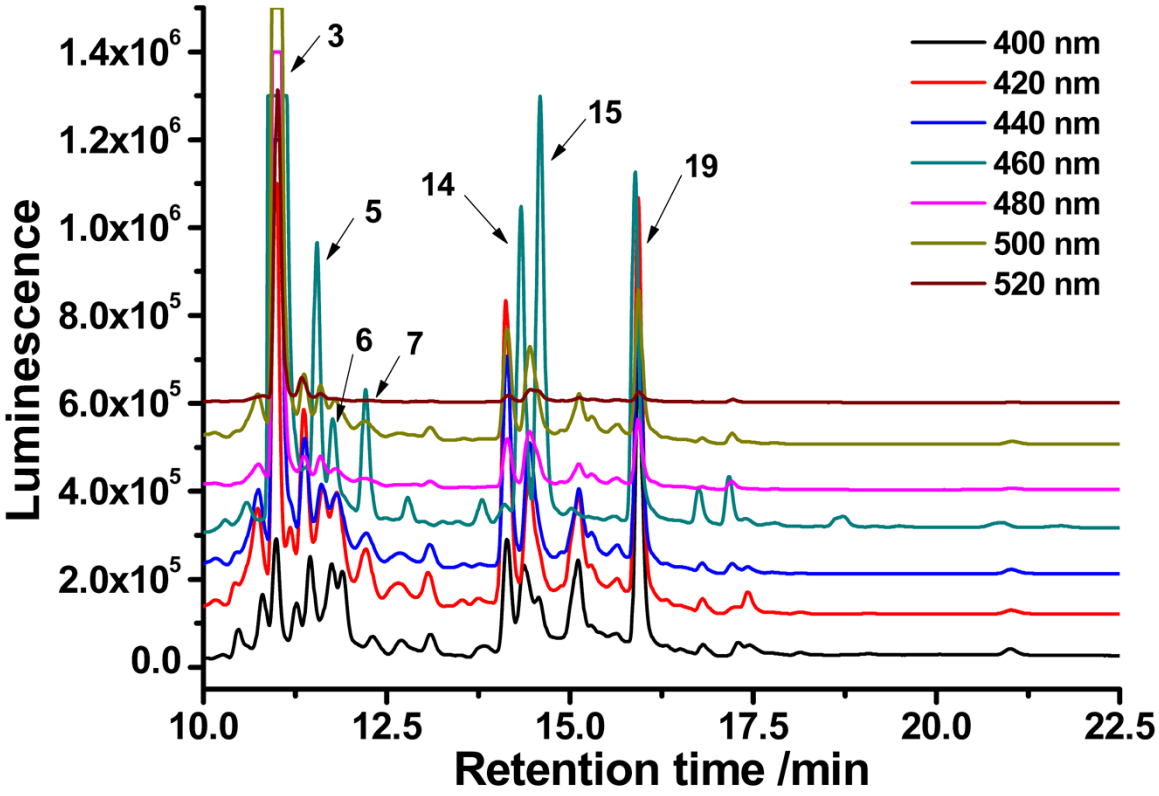

Fig. S-5. Chromatographic behavior of CQDs under different emission wavelengths. Flow velocity was 1.4 mL min^{-1}. The temperature was 25 ℃ and the excitation wavelength was 320 nm. The injection volume of the CQD samples was 2 μL (concentration, 0.5 mg mL^{-1}). The emission wavelengths were from 400 to 520 nm with an interval of 20 nm.

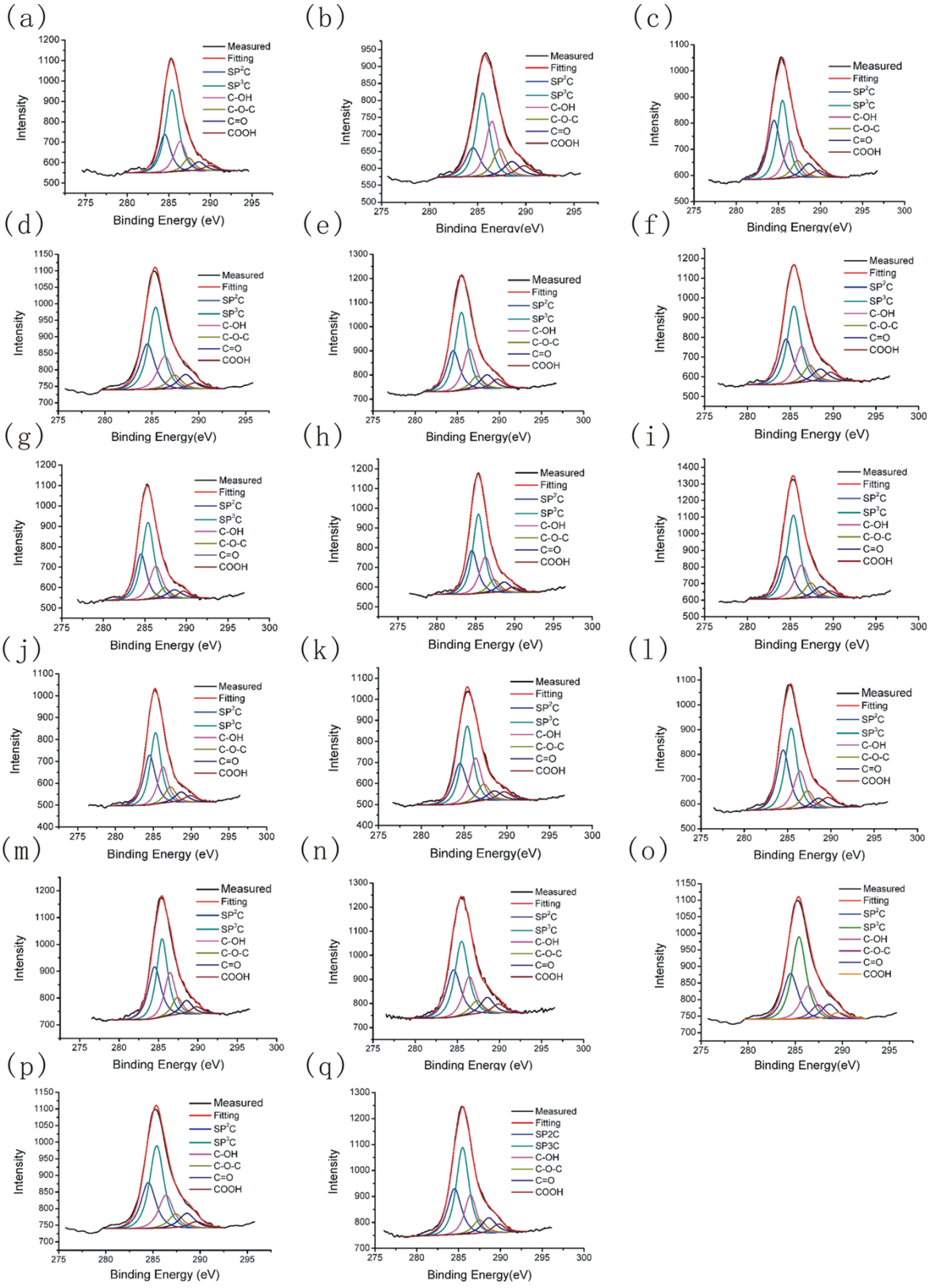
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(p)
(q)
Measured
Fitting
SP2C
SP3C
C-OH
C-O-C
C=O
COOH
Intensity
Binding Energy (eV)
Binding Energy(eV)

Fig. S-6. XPS spectrums of the seventeen collected fractions of CQDs. (a) ~ (q) were the XPS spectrums of seventeen fractions respectively. The relative percentages of functional groups of these fractions were in Table S-1. Seventeen fractions of CQDs were mentioned, including fraction 1, 3, 5-16, 19, 20, 21.

Table S-1. The chromatographic parameters of fraction 14 and 15 changed with the increase of flow rate

Flow rate/ mL min^{-1}	Fraction 14		Fraction 15		Resolution /R
	Retention time (min)/t_{R1}	Peak width /w_1	Retention time (min)/t_{R2}	Peak width /w_2	
0.60	18.99	0.24	19.19	0.34	0.69
0.80	17.09	0.26	17.29	0.28	0.74
1.00	15.87	0.23	16.05	0.25	0.75
1.20	14.89	0.23	15.12	0.23	1.00
1.40	14.33	0.23	14.59	0.23	1.13

Retention time and Peak width were provided by the LC solution of Shimadzu, the Resolution (R) of fractions 14 and 15 was calculated according to the following formula:

$$R = \frac{2(t_{R2} - t_{R1})}{W_1 + W_2}$$

Table S-2. The chromatographic parameters of fraction 14 and 15 changed with the increase of temperature

Temperature /℃	Fraction 14		Fraction 15		Resolution /R
	Retention time (min)/t_{R1}	Peak width /w_1	Retention time (min)/t_{R2}	Peak width /w_2	
25	14.34	0.23	14.59	0.23	1.09
30	14.18	0.24	14.39	0.24	0.88
35	14.04	0.25	14.21	0.25	0.68
40	13.85	0.23	13.94	0.25	0.38

Table S-3. The QY and the relative percentage of functional groups of the collected fractions

Fraction	The QY	The relative percentage of functional groups (%)					
		sp^2C	sp^3C	C-OH	C-O-C	C=O	-COO
1	0.011	20.43	48.44	16.58	6.59	5.17	2.79
3	0.76	14.76	36.37	23.39	12.12	7.88	5.48
5	0.032	29.48	34.68	16.61	7.86	7.61	3.76
6	0.031	25.02	40.10	17.26	6.96	6.32	4.34
7	0.032	21.58	39.98	19.93	6.32	7.72	4.47
8	0.003	23.44	39.39	17.64	7.88	7.06	4.59
9	0.003	23.71	43.18	18.70	5.44	5.44	3.53
10	0.003	23.54	41.29	19.43	6.69	6.21	2.84
11	0.003	22.39	42.96	17.44	7.32	6.42	3.47
12	0.027	27.74	36.65	17.60	8.20	5.96	3.85
13	0.097	21.80	38.39	21.05	8.43	5.56	4.77
14	0.091	27.72	35.71	17.56	7.99	4.96	6.06
15	0.059	27.13	35.85	19.34	7.48	6.68	3.52
16	0.023	25.50	37.89	18.00	6.28	7.89	4.44
19	0.022	24.63	35.59	21.71	7.61	6.18	4.28
20	0.009	23.99	41.36	16.62	6.90	7.82	3.31
21	0.006	23.58	41.30	16.81	6.39	7.97	3.95

Reference

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