Supporting information:

One-step Microwave Synthesis of Red Emissive Carbon Dots for Cells Imaging in Extreme Acidity and Light Emitting Diode

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Contents:

Experimental Section

1. Calculation of pKa Value.

The pKa value of R-CDs was calculated using the Henderson-Hasselbalch equation[1]:

$$\mathbf{pH=pKa+log\ ([A^-]/[HA])} \quad (1)$$

Where [HA] and [A-] are the molarities of the R-CDs acid and its conjugate base, respectively. According to the Lambert-Beer law, the concentration is directly proportional to the absorbance, which can be calculated by measuring the spectral intensity of the R-CDs at different pH values.

2. Cell Cytotoxicity Assay.

The cell viability assessment was carried out using the MTT assay.[2] A549 cells were cultured in RPMI 1640 culture medium supplemented with 10% FBS and 1% penicillin/streptomycin in the humidified 5% CO_2 incubator at 37°C.The cells were seeded in 96-well plates with a density of 5 × 10^5/mL cells and allowed to adhere overnight. After incubation with various concentrations of R-CDs (0-50 μg/mL) for 10 h at 37℃in a humidified atmosphere with 5% CO_2, 10 μL of MTT (5 mg/mL) was added to each well. After incubated for 4 h, the media were removed and 100 μL DMSO was added and shaken for 2 min, a micro-plate reader was used to measure the absorbance at 490 nm for determining cell viabilities. To avoid random errors, all the samples were performed in triplicate. The cell viability was calculated according to the equation:

$$\mathbf{Cell\ viability\ (\%) = [\sum(}A_i/A_{control}\mathbf{\times 100)]/n} \quad (2)$$

where A_i is the absorbance of different concentrations of R-CDs. $A_{control}$ is the average absorbance of the control well in which the probe R-CDs was absent, and n (=5) is the number of the data point.

3. Supplementary Figures.

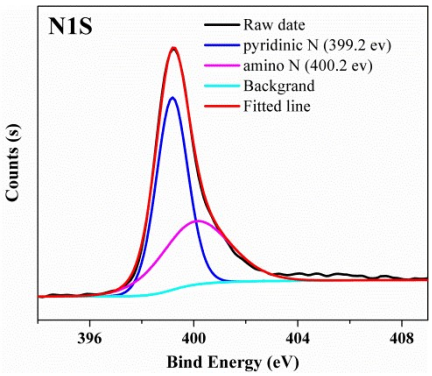


Fig. S1 The high-resolution XPS spectrum of N1s of 2, 3-diaminophenazine standard samples.

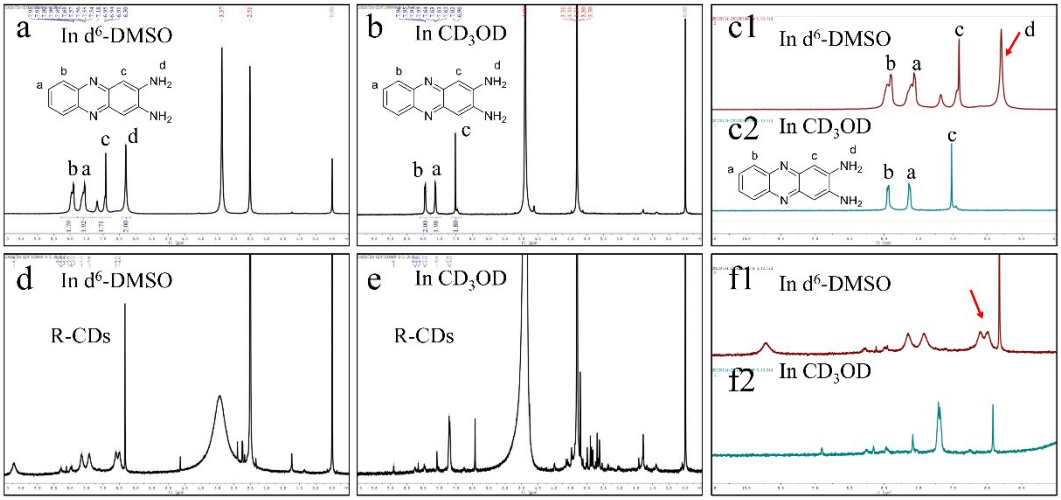


Fig. S2 ^{1}H-NMR of 2, 3-diaminophenazine in (a) d^{6}-DMSO and (b) CD_3OD. (c) Expanded spectrum at ppm 5.5-10.5 of a and b. ^{1}H-NMR of R-CDs in (d) d^{6}-DMSO and (e) CD_3OD. (f) Expanded spectrum at ppm 5.5-10.5 of d and e.

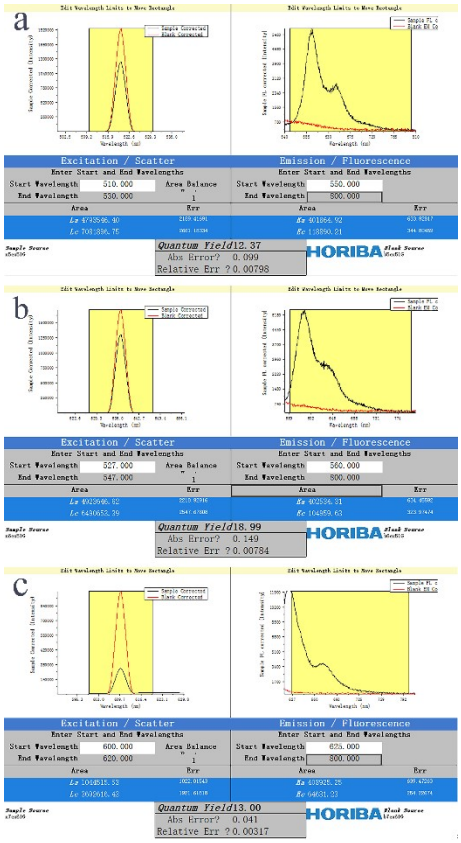


Fig. S3 The absolute fluorescence quantum yield (QY) of R-CDs in ethanol (a), in DMSO (b), and in PBS with pH=1 (c).

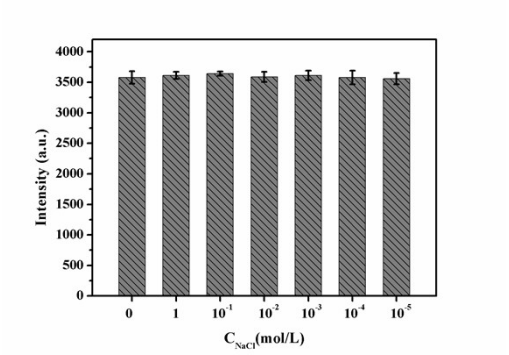


Fig. S4 Fluorescence intensity variation of the R-CDs under different ion strength (λex=611 nm).

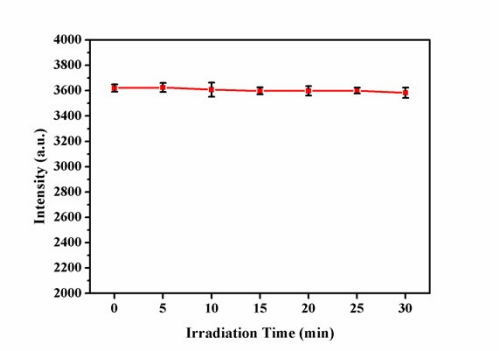


Fig. S5 Fluorescence intensity variation of the R-CDs at pH values of 1.0 with continuous UV irradiation for 30 min. (λex=611 nm).

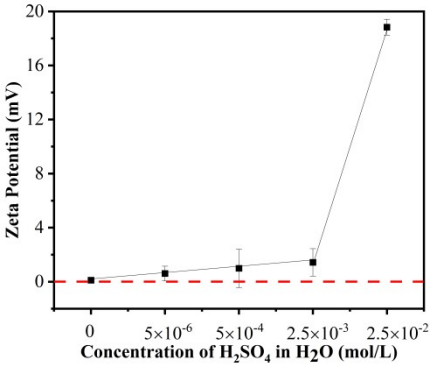


Fig. S6 The Zeta potential of R-CDs as a function of H_2SO_4 concentration.

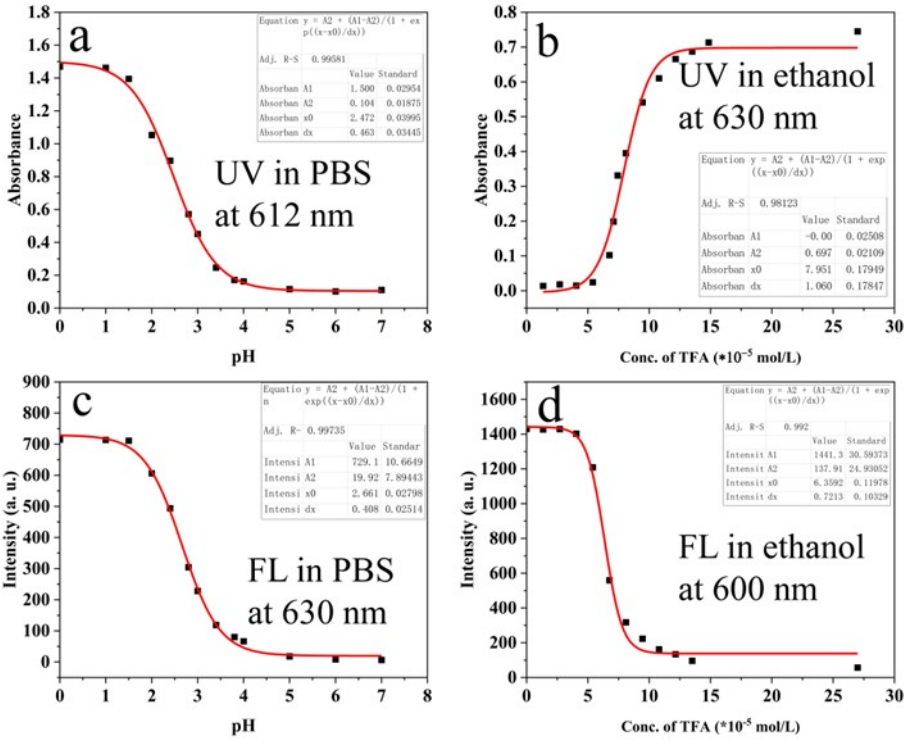


Fig. S7 UV-vis (a) and fluorescence emission spectra (c, λex = 630 nm) of R-CDs (10 μg/mL) in different pH buffer solutions and in ethanol with different concentration of TFA (b and d, λex = 600 nm).

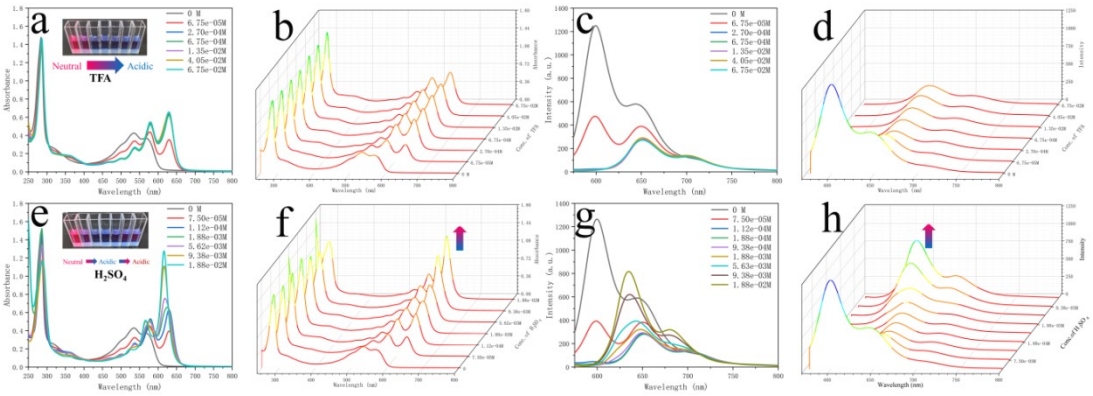


Fig. S8 UV-vis spectrum of R-CDs (10μg/mL) in ethanol as a function of TFA concentration (a and b). Fluorescence spectrum of R-CDs in ethanol as a function of TFA concentration (c and d). UV-vis spectrum of R-CDs in ethanol as a function of H_2SO_4 concentration (e and f). Fluorescence spectra of R-CDs in ethanol as a function of H_2SO_4 concentration (g and h).

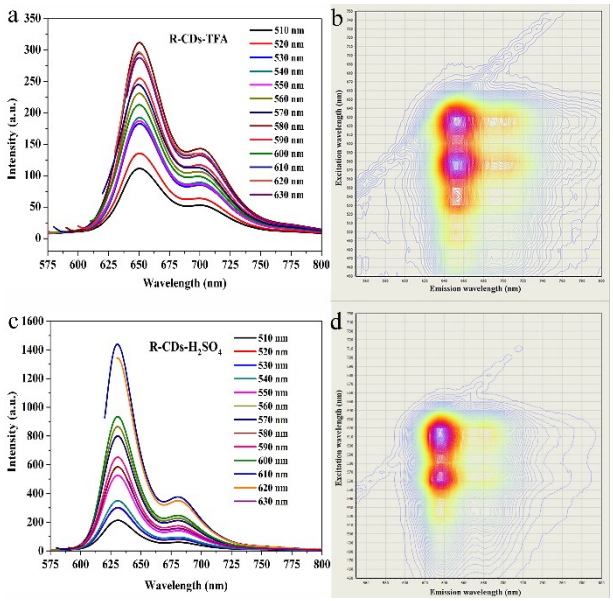


Fig. S9 Fluorescence emission spectra of R-CDs in TFA (a) and H_2SO_4 (b) under different excitation wavelengths. 3D fluorescence spectrum of the R-CDs in TFA (b) and H_2SO_4 (d).

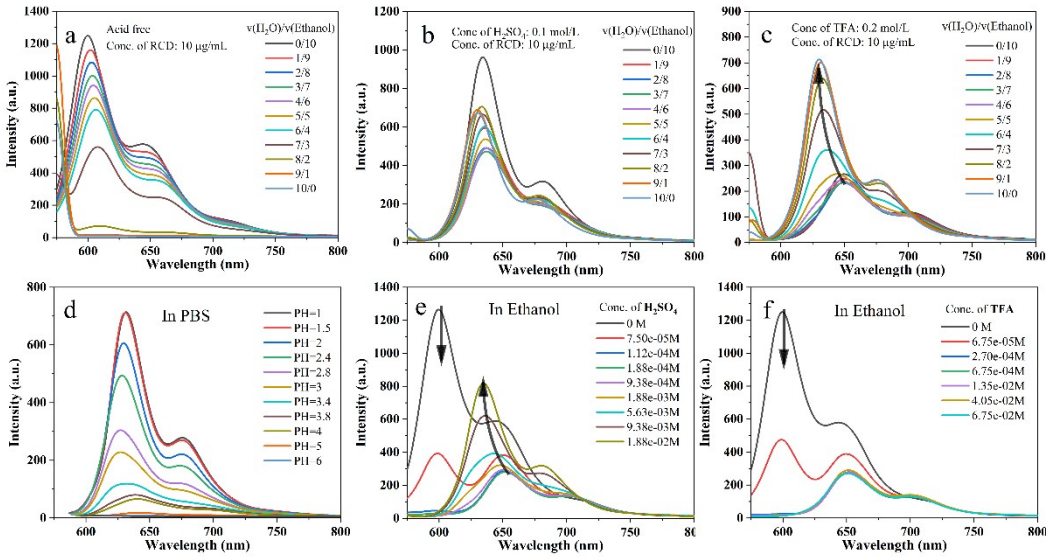


Fig. S10 Fluorescence spectrum of R-CDs as a function of VH2O/VEthanol with (a) acid free, (b) 0.1 mol/L H_2SO_4 and (c) 0.2 mol/L TFA. (Conc. of R-CDs: 10 μg/mL). (d) Fluorescence emission spectra of R-CDs in different pH buffer solutions. (e) Fluorescence spectra of R-CDs in ethanol as a function of H_2SO_4 concentration. (f) Fluorescence spectrum of R-CDs in ethanol as a function of TFA concentration.

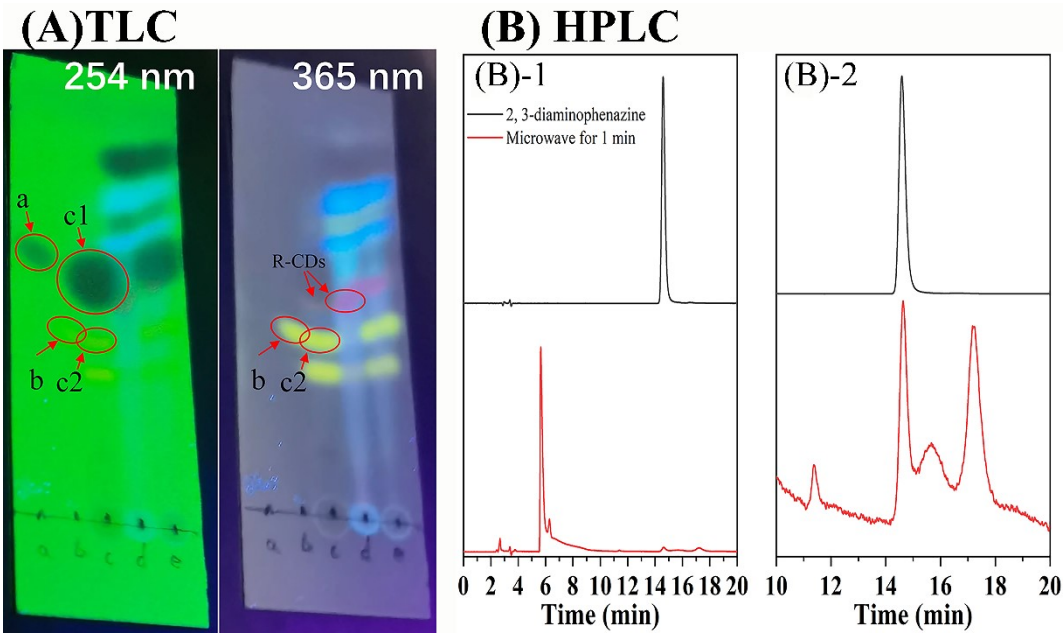


Fig. S11 (A) TLC of (a) o-PD, (b) 2, 3-diaminophenazine standard sample, products under microwave reaction for (c) 1 min, (d) 4 min, and (e) the mixture of a-d. λex = 254 nm (Left)/ 365 nm (Right). (B) HPLC of 2, 3-diaminophenazine (black line) and the product after microwave of o-PD for 1 min (red line). (B)-1: Time 0-20min. (B)-2: Time 10-20min.

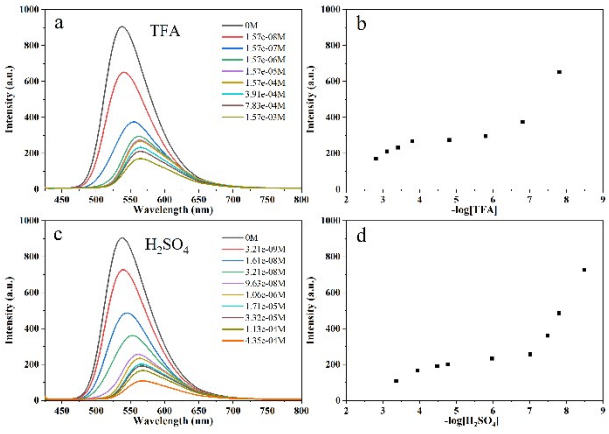


Fig. S12 (a) Fluorescence spectrum of DAP in ethanol as a function of TFA concentration. (b) Fluorescence intensity of DAP in ethanol as a function of –log[TFA]. (c) Fluorescence spectra of DAP in ethanol as a function of H_2SO_4 concentration. (d) Fluorescence intensity of DAP in ethanol as a function of –log[H_2SO_4].

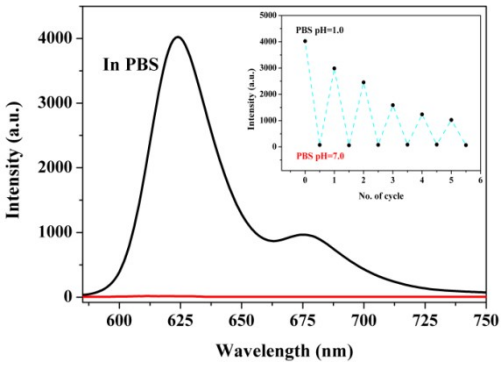


Fig. S13 Fluorescence intensities of R-CDs (10 μg/mL) in PBS (pH=1.0) and PBS (pH=7.0) during 6 cycles of transformation. λex = 600 nm.

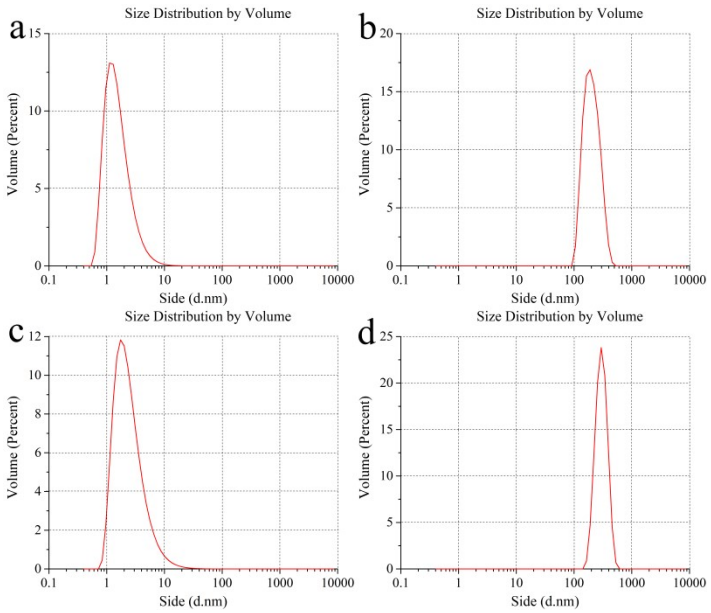


Fig. S14 The particle size of R-CDs in ethanol at pH=5 (a) and pH=7(c), and the particle size of R-CDs in ethanol at pH=5 (b) and pH=7 (d) after 6 cycles.

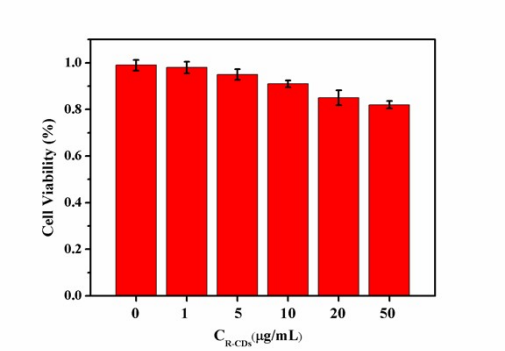
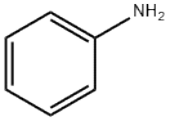
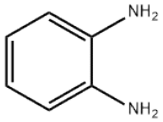
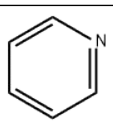
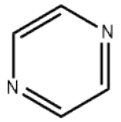
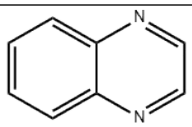
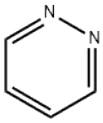
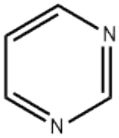
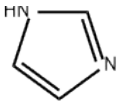


Fig. S15 Cell viability of A549 cells treated with different concentrations of R-CDs.

Table S1 The pKa of nitrogen in different structure.[3]

No.	Name	Structure	pKa
1	Anilin		4.63
2	1,2-Diaminobenzene		4.46
3	Pyridine		5.25

4	**Pyrazine**		**0.65**
5	**Quinoxaline**		**0.56**
6	**1,2-Diazabenzene**		**2.24**
7	**1,3-Diazabenzene**		**1.23**
8	**1,3-Diazole**		**6.95**

Reference

1. H. N. Po and N. M. Senozan, *J. Chem. Educ.*, 2001, **80**, 146.
2. W. Niu, L. Fan, M. Nan, Z. Li, D. Lu, M. S. Wong, S. Shuang and C. Dong, *Anal. Chem.*, 2015, **87**, 2788-2793.
3. https://www.cas.org/products/scifinder OR https://www.chemicalbook.com/