A facile fluorescence method for the effective detection of ampicillin using antioxidant carbon dots with specific fluorescent response to ·OH

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S1. Experimental sections

S1.1. Reagents and apparatus

Citric acid, ethylenediamine, 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt) (ABTS), sodium chloride (NaCl), zinc chloride ($ZnCl_2$) and streptomycin (SM) were purchased from Aladdin Biochemical Technology Co., (Shanghai, China). Erythromycin (EM), vancomycin (VA), chloramphenicol (CPL), ampicillin (AMP), penicillin G (PG) and 2,2-diphenyl-1-pyridine hydrazyl (DPPH) were provided by Macklin Biochemical Co., (Shanghai, China). 30% hydrogen peroxide solution (H_2O_2) was purchased from Xilong Science Co., (Shantou, China). Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium chloride (KCl) and glucose were purchased from Sinopharm Chemical Reagent Co., (Shanghai, China).

Ultraviole-visible absorption spectra were recorded with UV-2450 spectrophotometer (Shimadzu Corporation, Japan). Fourier transform infrared spectroscopy (FTIR) was collected by NICOLET iS50 Infrared Spectroscopy (Thermo Fisher Scientific, USA). Water contact angle was determined using Theta Lite optical contact angle meter (Biolin scientific, Sverige). Transmission electron microscopy (TEM) images were performed on a FEI Talos F200S (Thermo Fisher Scientific, USA). Fluorescence spectra were recorded on Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA).

S1.2. Quantum yield (QY) measurement

The relative fluorescence quantum yield of E-CDs ($QY_{E\text{-}CDs}$, Ex=345 nm) was calculated

using quinine sulfate (Qs=54.6%) in 0.1 mol/L H_2SO_4 solution as a reference. The absorbance of the aqueous solution of E-CDs with quinine sulfate was measured at 350 nm, and the absorbance value of the solution was kept below 0.05 to minimize self-absorption. And the fluorescence spectra of the above solutions were determined at 350 nm excitation. The $QY_{E\text{-}CDs}$ were calculated using the following equation.

$$QY_{E-CDs} = QY_{Qs}\frac{K_{E-CDs}}{K_{Qs}}\left(\frac{\eta_{E-CDs}}{\eta_{Qs}}\right)^2$$

$QY_{E\text{-}CDs}$ and QY_{QS} are the quantum yields of E-CDs and quinine sulfate, respectively. η is the refractive index (1.33 for water and 0.1 M H_2SO_4). K is the slope of the integrated fluorescence intensity-absorbance curve.

S1.3. Measurement of ESR

Verification of the reaction between AMP and •OH was conducted using electron paramagnetic resonance spectroscopy. 1.4 mL of PBS, 200 μL of $FeSO_4$ (5 μM), 200 μL of H_2O_2 (500 μM) and 200 μL of AMP (50 μg/mL) were mixed. After incubation for 4 or 16 minutes, the EPR spectra were measured by adding DMPO.

The Stern-Volmer equation of $F_0/F=1+Ksv[Q]$ was used to observe the change of the quenching constant after the temperature of the reaction system increased[1] (where F_0 and F are the fluorescence intensity of the fluorescence substance without and with the quenching agent, respectively; [Q] is the concentration of quencher; Ksv is the dynamic quenching constant).

S2. Supplementary Figures

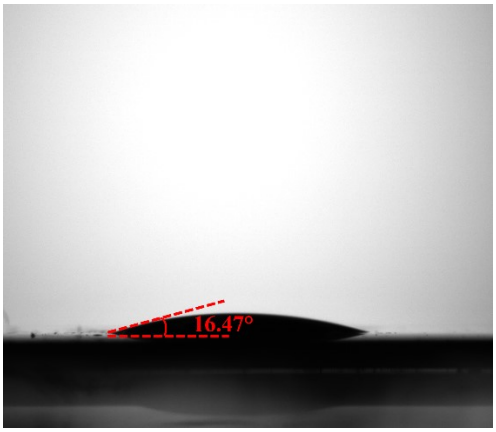

Fig. S1. The water contact angle of the E-CDs

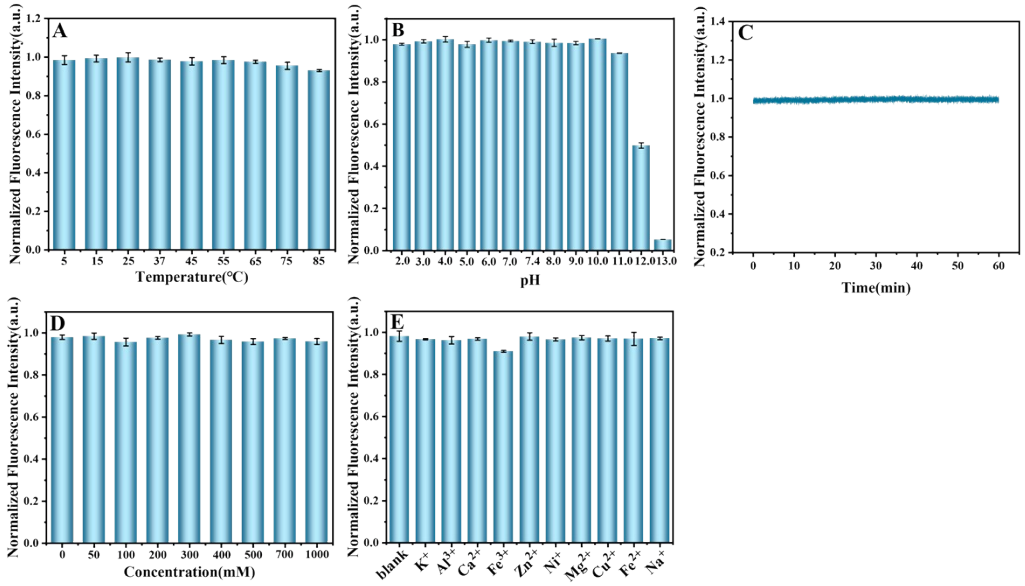

Fig. S2. Fluorescence responses of E-CDs at different temperatures (A), variable pH values(B), continuous exciting at 350 nm for 1 hour (C), different NaCl concentrations (D), and to different metal ions (E).

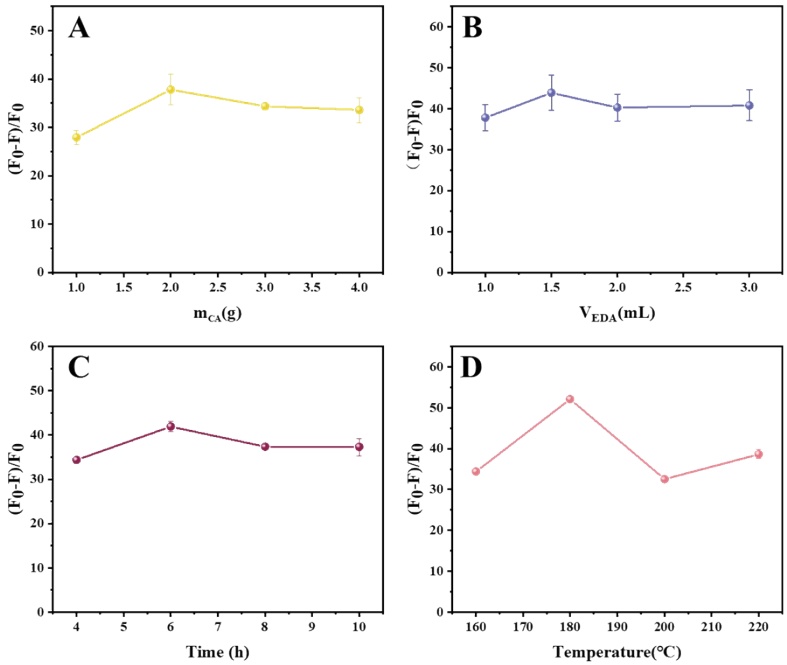

Fig. S3. The response capability of CDs synthesized with different citric acid content (A), different ethylenediamine content (B), different reaction time (C), and different reaction temperature (D) to •OH.

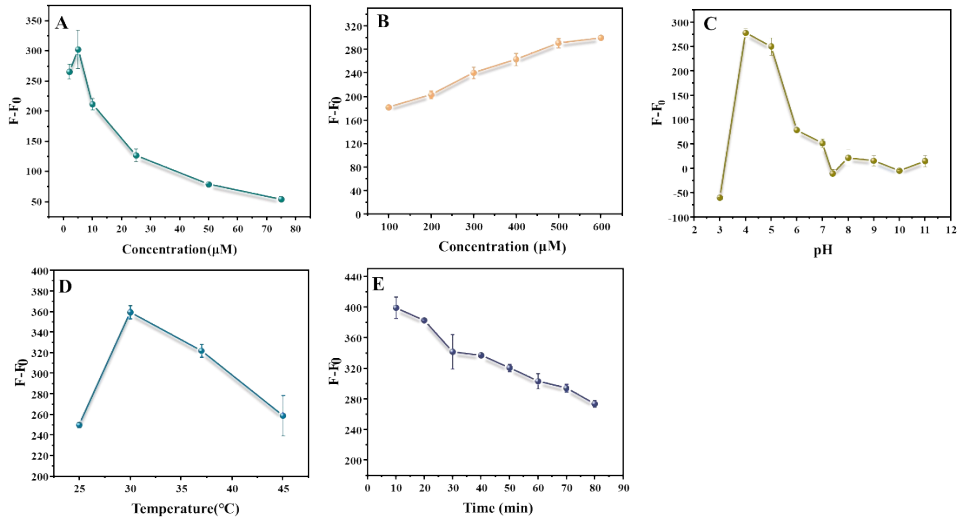

Fig. S4. The effects of Fe^{2+} concentration (A), different H_2O_2 concentrations (B), pH (C), reaction temperature (D), and reaction time (E) on $F\text{-}F_0$.

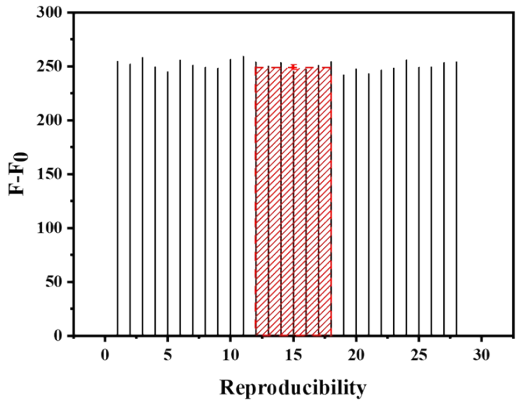

Fig. S5. The repeatability of the detection method (n=28).

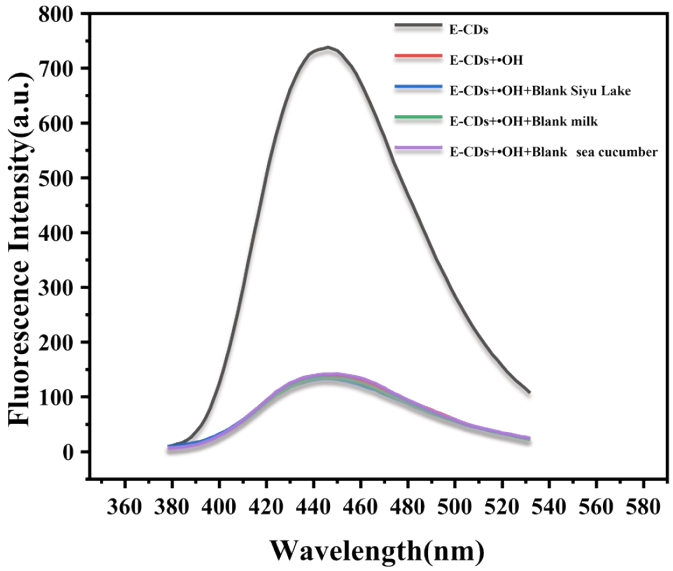

Fig. S6. Fluorescence spectra of the E-CDs+H_2O_2+Fe^{2+} system after reacting with Siyu Lake water, milk, and sea cucumber samples with the same treatment before the addition of AMP.

S3. Supplementary Tables

Table S1 QY of different antioxidant CDs

Sample	λ_{ex} (nm)	QY(%)	References
E-CDs	350	81.87	This work
Se-CDs	379	7.10	2
R-CDs	560	14.00	3
L-CDs	370	10.00	4
Se-CDs	360	8.30	5
ASAC-CDs	353	3.17	6
ClCQDs	440	28.40	7

Table S2 Comparison of the performance of AMP sensors with other sensors and this work

Probe	LOD (μg/mL)	Detection range(μg/mL)	Reference
EuNS	1.74	0-17.43	8
Ni^{2+}	0.52	17.47-69.88	9
CDs	0.24	0-20.96	10
CND	5.81	6.6-200	11
AuNPs	13.00	16-96	12
AgNPs	0.01	0.025-1.2	13
E-CDs	0.38	0.5-20 and 20-80	This work

Table S3 Testing results of ampicillin capsules(n=3)

AMP（μg/mL）	Specifications（mg/grain）	Tested result (μg/mL)	Tested result (mg/grain)	Labeled percentage (%)	RSD (n=3, %)
6	250	5.95	247.99	99.20	0.39
10		9.89	247.28	98.91	0.64

Supplementary References

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