

A Copper-Mediated Fenton-Like Dual-Mode Optical Sensor for Sensitive Determination of Acetylcholinesterase Activity in Biological Fluids

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2.2. Instrumentation

The optical characterization of the synthesized carbon dots was probed through fluorescence and UV-Vis spectroscopic methods, wherein the fluorescence emission spectra were acquired on a Shimadzu RF-5301PC spectrofluorometer by exciting the samples at 310 nm with 5 nm slit widths, while the absorption spectra were generated using a Shimadzu 1601 PC UV-Vis spectrophotometer. The structural and morphological aspects were elucidated by transmission electron microscopy using a JEOL 2100F instrument operating at 200 kV accelerating voltage to visualize the size and shape of the carbon dots, X-ray diffraction patterns recorded with a Philips PW 1700 diffractometer, and Fourier transform infrared spectroscopy performed on KBr pellets using a Nicolet 6700 spectrometer. Furthermore, the surface chemical composition and bonding states were evaluated through X-ray photoelectron spectroscopy utilizing an ESCALAB250 spectrometer from Thermo Scientific.

2.4. Quantum yield determination of N, S-CDs

The quantum yield (QY) of N, S-CDs in phosphate buffer was calculated according to the following equation using quinine sulfate (QS) as a reference in 0.1 M H₂SO₄ (QY = 54 %). By measuring the absorbance (less than 0.05) and emission spectra of a certain concentration of N, S-CDs and quinine sulfate at the excitation wavelength at 360 nm, the absorbance and fluorescence integral area were substituted into the following formula:

$$\phi_{N,S-CDs} = \phi_{QS} \times \frac{F_{N,S-CDs}}{F_{QS}} \times \frac{A_{QS}}{A_{N,S-CDs}} \times \frac{\eta_{N,S-CDs}}{\eta_{QS}}$$

$\Phi_{N,S-CDs}$ represents the quantum yield of N, S-CDs, ϕ_{QS} represents the quantum yield of QS, $F_{N,S-CDs}$ is the fluorescence intensity of N, S-CDs, F_{QS} is the fluorescence intensity of quinine sulphate,

A refers to the absorbance value and η refers to the refractive index of the solvent (double distilled water). The synthesized N, S-CDs were dissolved in distilled water ($\eta = 1.33$) and quinine sulfate was dissolved in 0.1 M H_2SO_4 ($\eta = 1.33$).

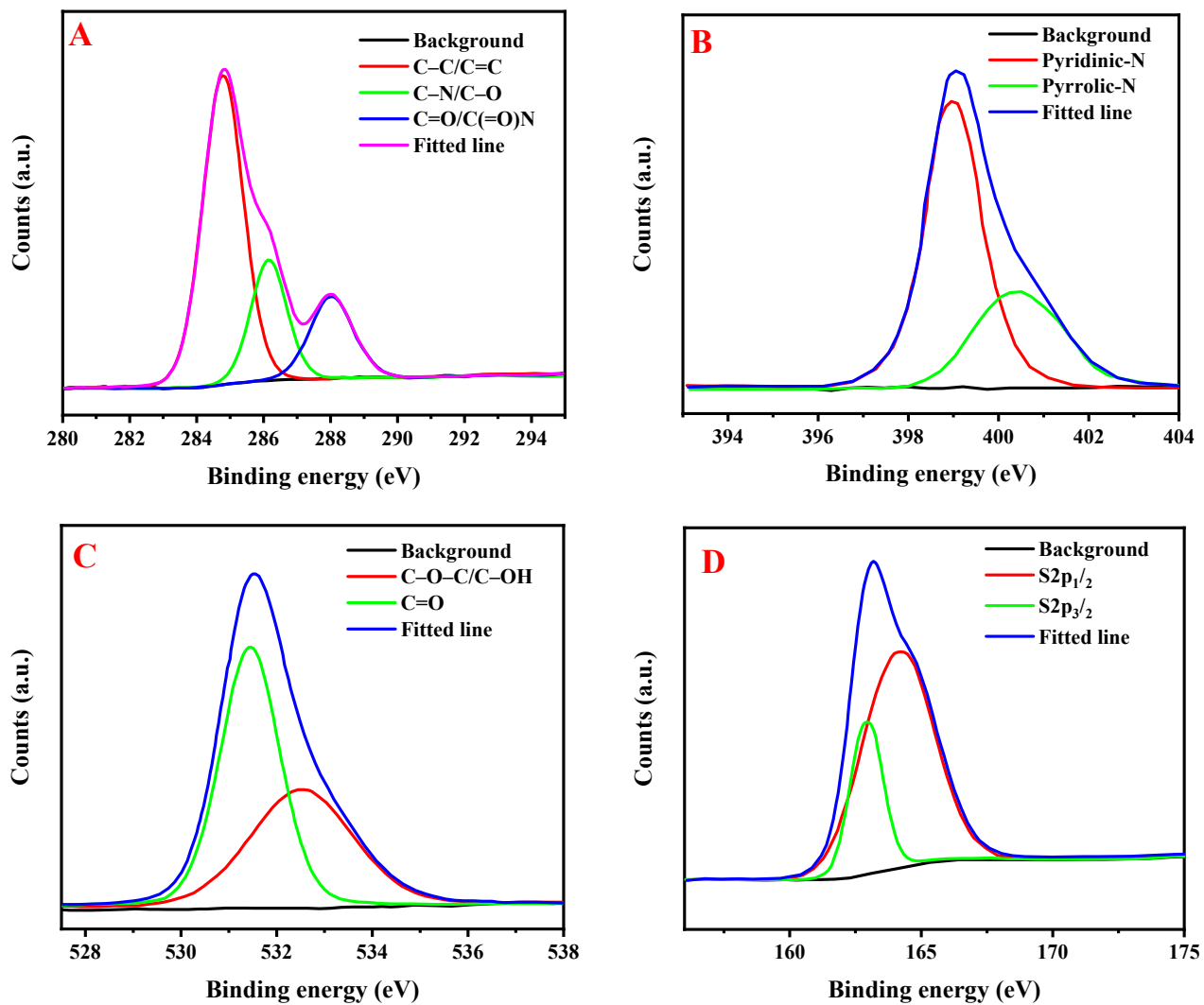


Fig. S1. Detailed XPS spectra of (A) C1s, (B) N1s, (C) O1s and (D) S2p of N, S-CDs.

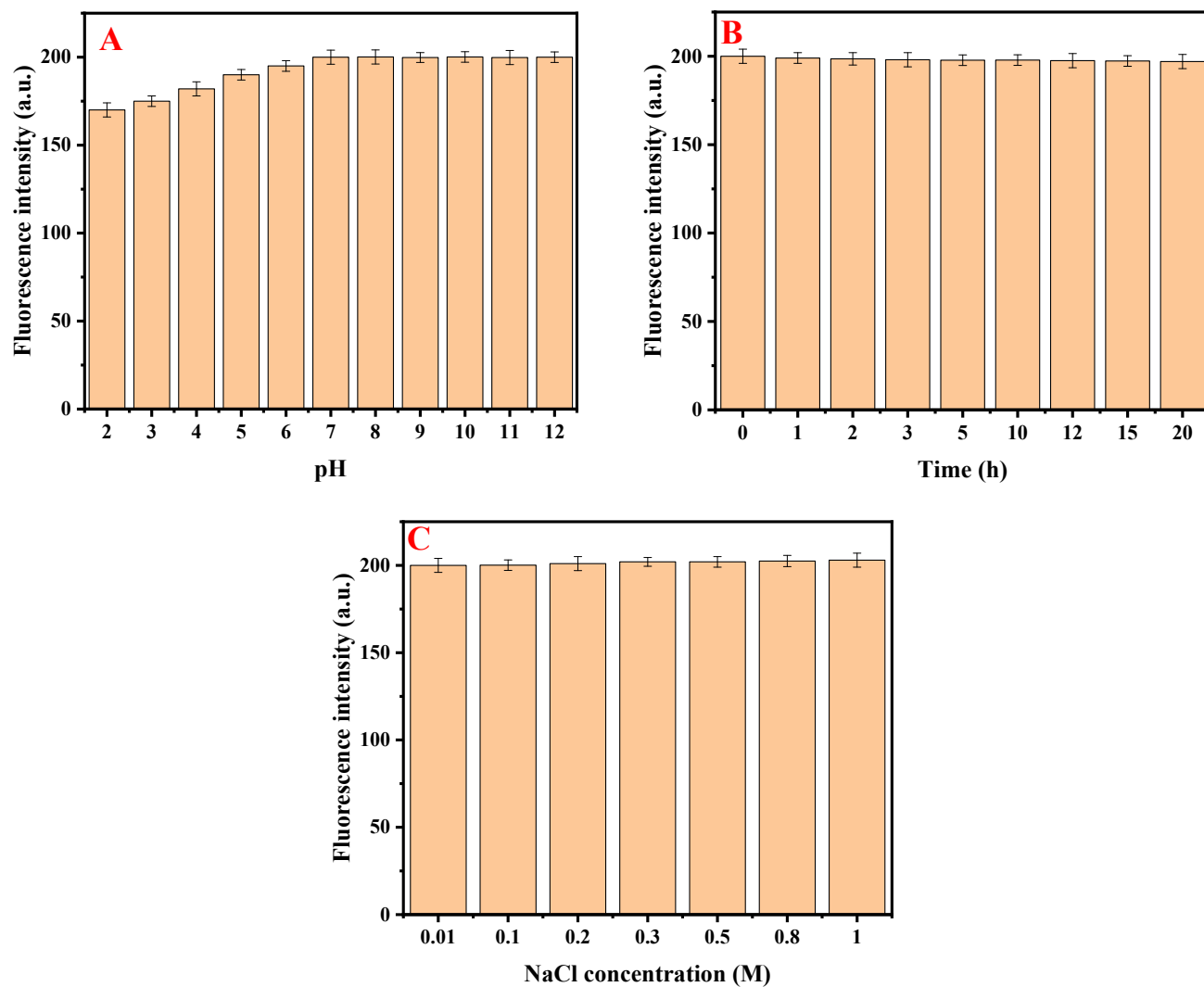


Fig. S2. Stability of the fluorescence response of the prepared N, S-CDs under various conditions: (A) effect of pH (2 – 12), (B) effect of UV irradiation time (0 –20 h) (C) effect of NaCl concentration (0.01 – 1.0 M) and. All measurements were performed in triplicates.

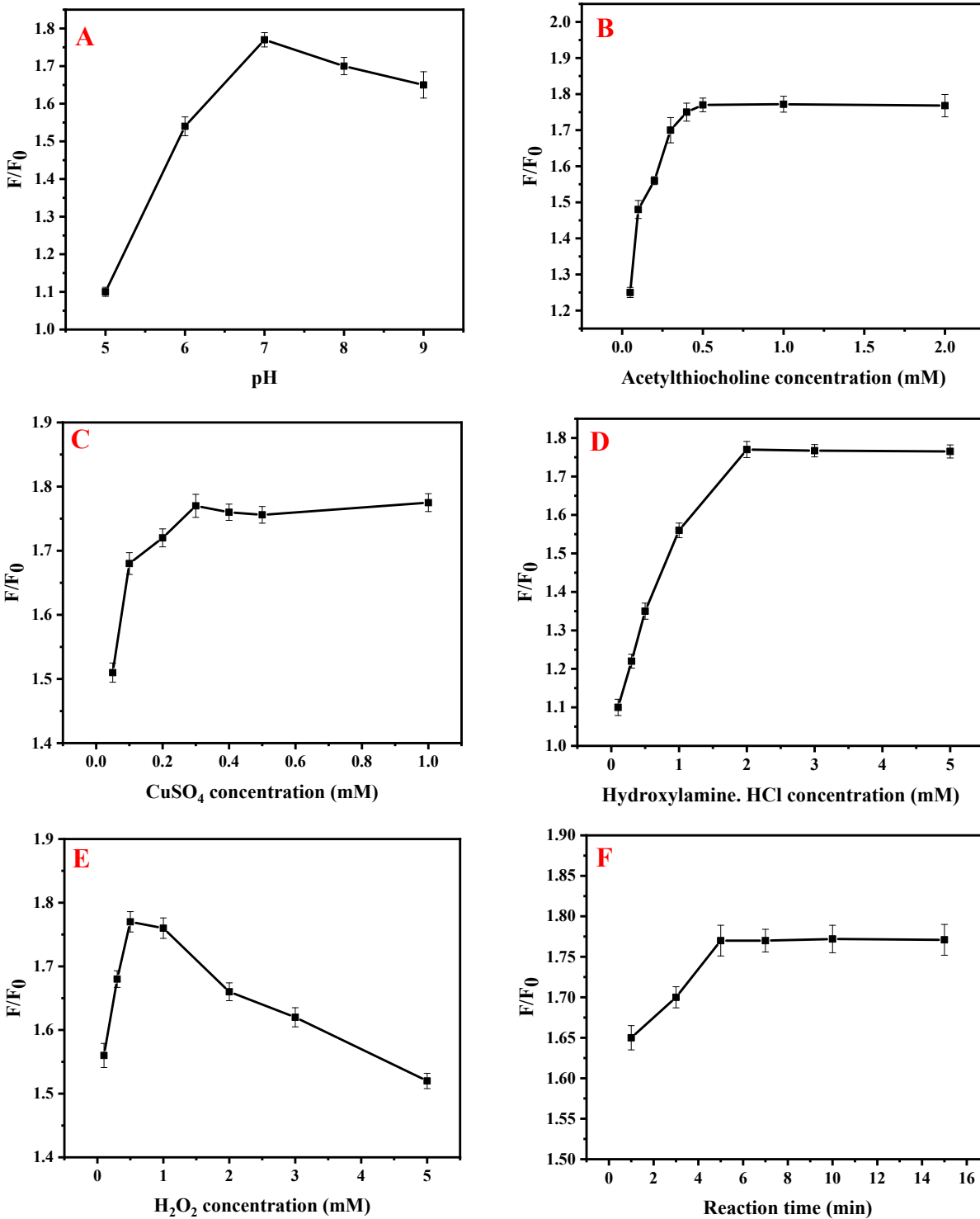


Fig. S3. Effect of (A) pH (5.0–9.0), (B) acetylthiocholine concentration (0.05–2.0 mM), (C) CuSO_4 concentration (0.05 to 1.0 mM), (D) hydroxylamine. HCl concentration (0.1–5.0 mM), (E) H_2O_2 concentration (0.1–5.0 mM), and (F) incubation time (1–15 min.) on F/F_0 of $\text{Cu(I)/H}_2\text{O}_2/\text{N}$, S-CDs.

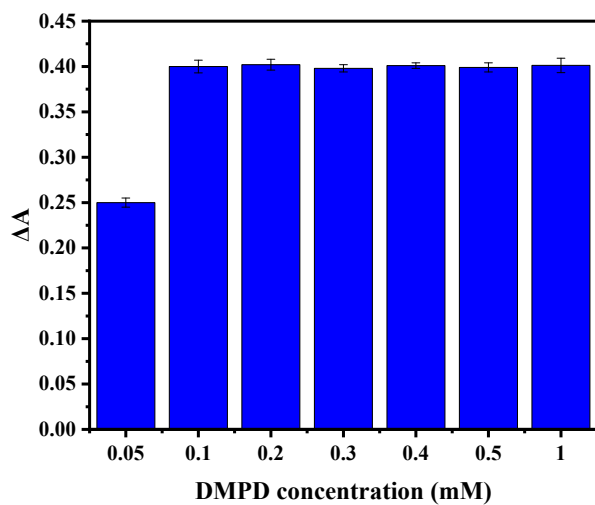


Fig. S4. Effect of DMPD concentration (0.05 and 1.0 mM) on the absorbance of Cu(I)/H₂O₂/DMPD

Table S1. Comparison of the proposed method for AChE detection with previously reported methods.

Method	Sensor	Linearity range (mU/mL)	LOD (mU/mL)	Ref.
Colorimetric	Au@PDA NPs hydrogel	2.5–25	0.9	1
	Fe-N-C SAzymes	0.5–25	0.014	2
	FeMn DSAs/N-CNTs	0.1–30	0.066	3
	Cu-Fe-ZIF-8/P-GCNN-H ₂ O ₂ -TMB	0.004–0.02	0.00013	4
	Phosphorus quantum dots	0.5–10	0.17	5
	Cu(I)/H ₂ O ₂ /DMPD	0.1–10.0	0.028	This work
Fluorometric	Ag-AuNCs@SiNPs nanohybrids	0.03–4.0	0.0021	6
	Cysteine-sensing system	0.05–1.3	0.05	7
	gold nanoclusters-Cu ²⁺	0.05–25	0.05	8
	AuAgNCs	0.4–25	0.15	9
	ATCI-H ₂ O ₂ -OPD	0.01–8	0.006	10
	GSH-Au NCs@ZIF-8	0-5	0.017	11
	Cu(I)/H ₂ O ₂ /N, S-CDs	0.02–0.6	0.0068	This work

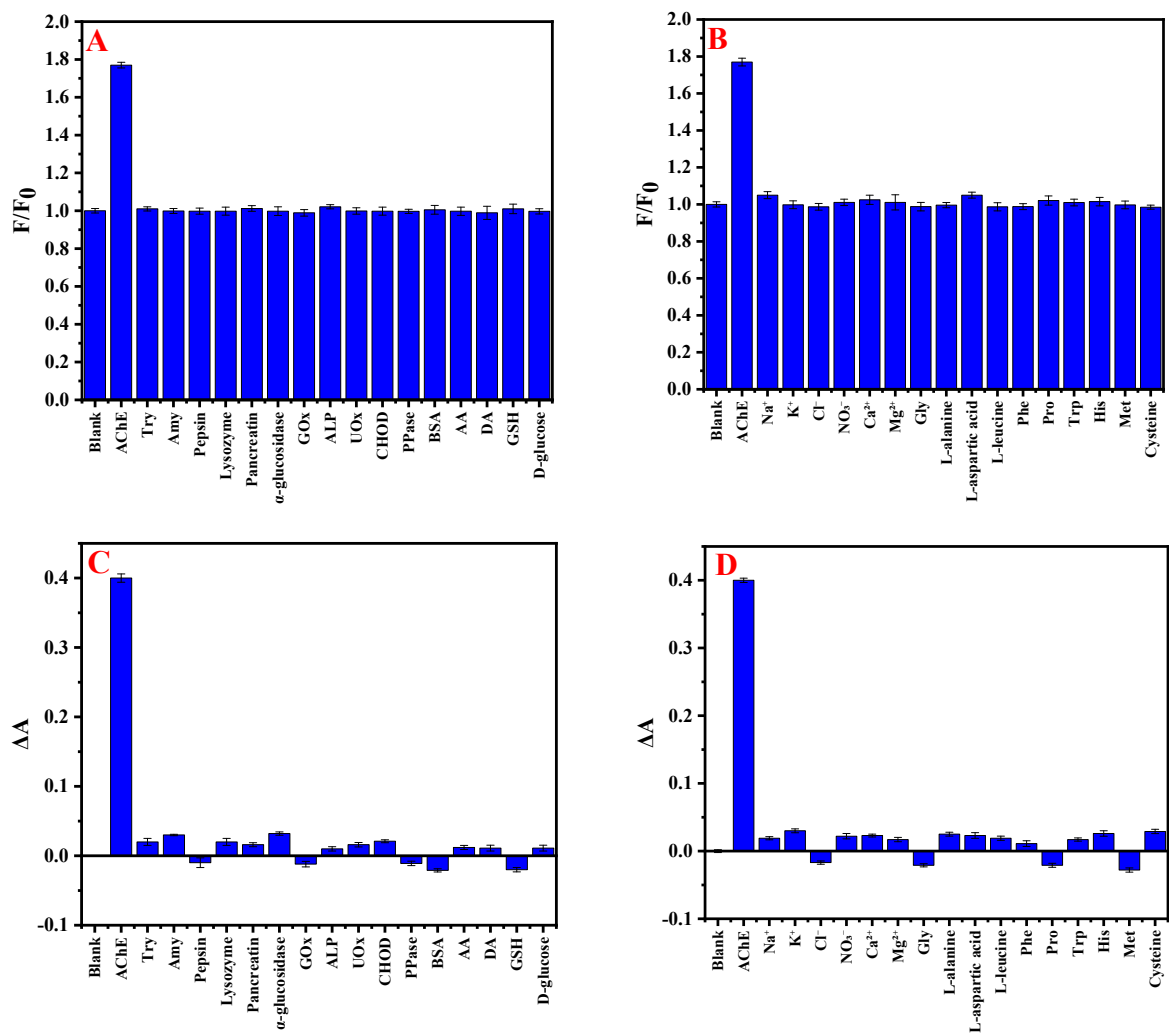


Fig. S5. Effect of AChE and different interferants on (A) F/F₀ of Cu(I)/H₂O₂/N, S-CDs and (B) ΔA of Cu(I)/H₂O₂/DMPD system.

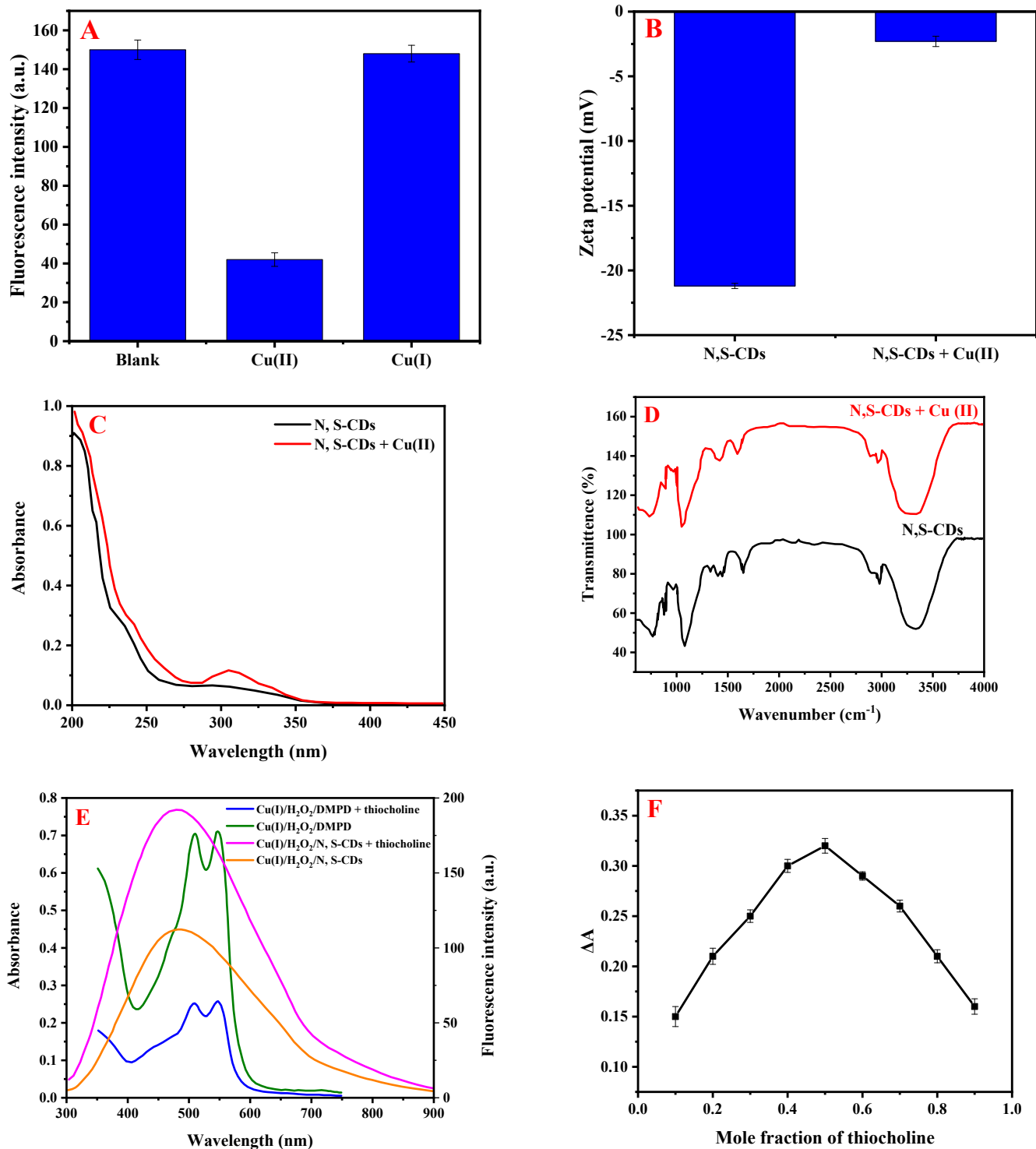


Fig. S6. (A) Fluorescence emission of H₂O₂/N, S-CDs in presence of Cu(II) and Cu(I), (B) zeta potential of N, S-CDs before and after addition of Cu(II), (C) absorption spectrum of N, S-CDs before and after addition of Cu(II), (D) FTIR spectrum of N, S-CDs before and after addition of Cu(II), (E) emission spectrum of Cu(I)/H₂O₂/N, S-CDs and absorption spectrum of Cu(I)/H₂O₂/DMPD before and after addition of thiocholine, and (F) Job's plot for thiocholine-Cu(I) interaction.

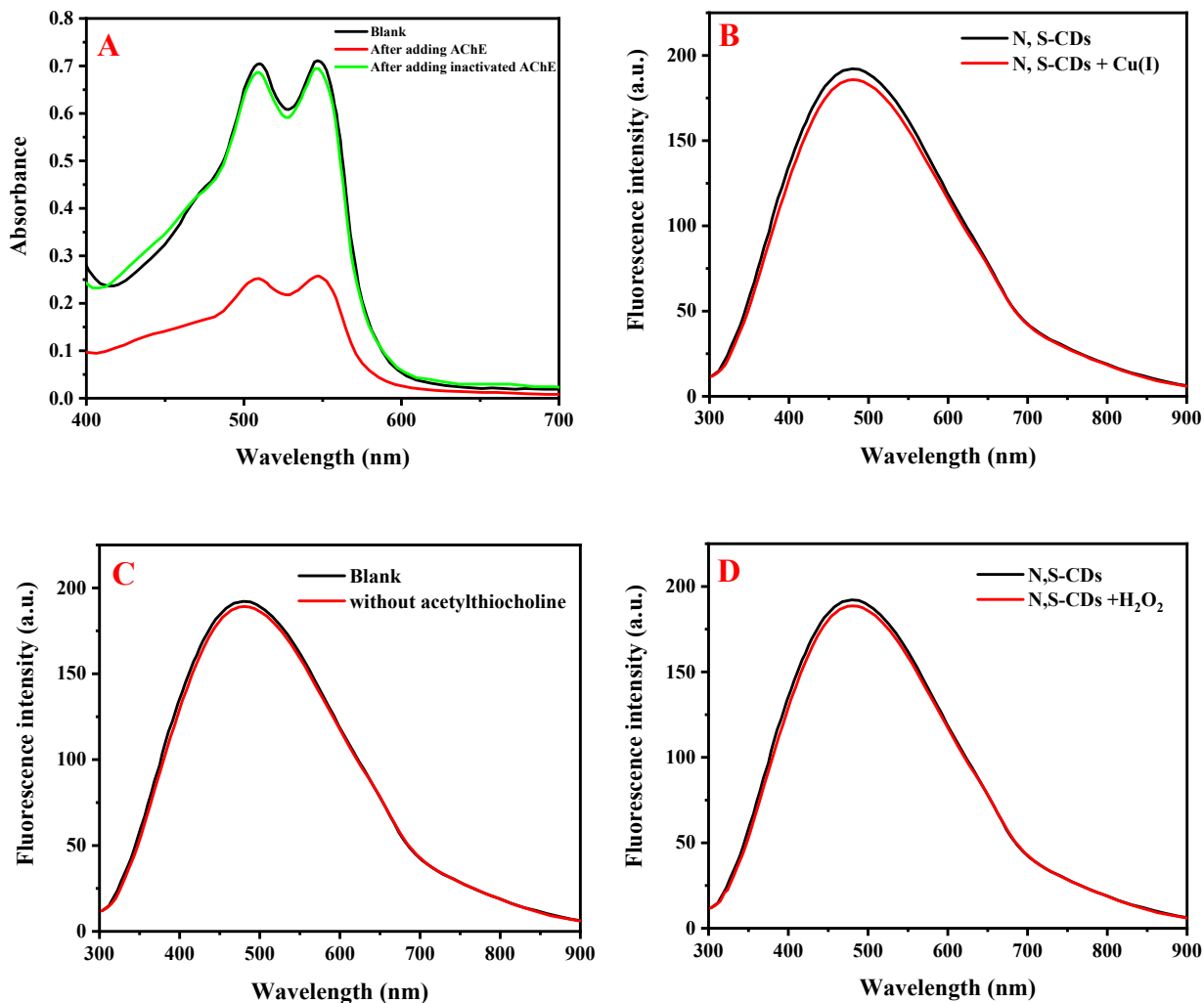


Fig. S7. (A) absorption spectrum of Cu(I)/H₂O₂/DMPD after addition of AChE and inactivated AChE, (B) Fluorescence emission of N, S-CDs in presence of Cu(I), (C) Fluorescence emission of Cu(I)/H₂O₂/N, S-CDs in absence of acetylthiocholine, and (D) Fluorescence emission of N, S-CDs before and after addition of H₂O₂.

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