Facile synthesis yellow fluorescent carbon dots for highly sensitive sensing cobalt ions and biological imaging

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Experimental

Materials

O-phenylenediamine, urea, $Al_2(SO_4)_3$, $CdSO_4 \cdot 8H_2O$, $CoSO_4 \cdot 7H_2O$, $Cr_2(SO_4)_3 \cdot 6H_2O$, $FeSO_4$, $FeCl_3 \cdot 6H_2O$, $HgCl_2$, KCl, $MgCl_2 \cdot 6H_2O$, $MnSO_4 \cdot H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2 \cdot 6H_2O$, $CuSO_4.5H_2O$, 2,4-dichlorophenol, phenol, benzyl alcohol, benzoic acid, m-nitrobenzoic acid, o-nitrobenzoic acid, o-nitrotoluene, nitrobenzene, ammonium nitrate, ethyl alcohol, Rhodamine 6 G were all analytical grade. O-phenylenediamine was purchased from Tianjin Zhiyuan Chemical Reagent Co, Ltd. Other chemicals were obtained from Aladdin (Shanghai, China). High purity water with a resistivity of 18.2 MΩ cm was obtained from Molelement element ultra pure water machine. Dialysis bag (1000 Da) was purchased from Shanghai Chemical Reagent company (Shanghai, China). B-Complex B-12 (250 mg/tablet) was purchesed from guoda

drugstore. Tap water was obtained from ShanXi University. *Zebrafish* were collected from School of Life Sciences, Shanxi University.

Characterization

The morphology and structure of the N-CDs were analyzed by a transmission electron microscope (TEM) (JEOL, JEM-2100), operating at 200 kV) (Tokyo, Japan). The Fourier transform infrared spectra of the N-CDs was performed using Nicolet iS50 FT-IR spectrometer (Thermo Scientific. USA). X-ray photoelectron spectroscopy analysis were acquired on an Escalab 250Xi electron spectrometer (Thermo Fisher Scientific, USA) using monochromatic Al Ka radiation. UV-vis absorption spectra were collected by a Shimadzu Corporation UV-2450 Spectrophotometer with a 1 cm sample cell. Steady-state fluorescence spectra were obtained on a Shimadzu Corporation RF-5301 Spectrophotometer (Tokyo, Japan). The fluorescence lifetimes were taken on a PTI QuantaMaster™400 and PicoMaster 1000-TCSPC spectrofluorometer. ZEISS LSM 880 confocal laser scanning microscope was employed for biological imaging.

Quantum yield measurement

The quantum yield (QY) of N-CDs was calculated by comparing the fluorescence intensities and absorption values of N-CDs solution with

Rhodamine 6 G (excitation wavelength: 488 nm, quantum yield 0.94, dissolved in ethanol). In order to minimize re-absorption effect, the absorbance of the N-CDs solution was kept below 0.05. The QY was measured based on the following equation:

$$Q_C = Q_R * I_C/I_R * A_R/A_C * (n_C/n_R)^2$$

where Q is the QY, I refers to the integrated emission intensity, A is the absorbance at excitation wavelength, and n represents the refractive index of the solvent. The subscript "R" and "C" stand for standard with known QY and the sample, respectively.

Toxicity assays

The *Zebrafish* were cultured in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 5 – 10% methylene blue; pH 7.5) at 28 ℃ for 4 days. After that, the N-CDs powder were configured into 6 gradient concentrations with E3 embryo media (12.5, 25, 50, 100, 200, 300 mg mL^{-1}, respectively). Then, putting each concentration N-CDs solution (5 mL) and *Zebrafish* (10 pieces) into culture dish successively and incubating for 24 h. Finally, calculating the semi-lethal concentration by mortality data, and the corresponding concentration with a mortality rate of less-than 8% was selected for imaging experiments.

Biological imaging

The *Zebrafish* (4 days old) were interacted with N-CDs and N-CDs+Co^{2+} for 1 h at 28℃, respectively. Then, the *Zebrafish* was further incubated with HSO_3^- (200 μM) for 1 h. After washed with PBS, the *Zebrafish* were imaged by a ZEISS LSM 880 confocal laser scanning microscope.

Results and Discussion

Stability of N-CDs

To explore the assay conditions of the N-CDs employed as a fluorescent probe in Co^{2+} detection, we optimized some analytical conditions. First, the pH-dependence of the N-CDs solutions was determined by measuring the fluorescence intensities over a pH range. The fluorescence intensity was maximized at pH=8, so pH=8 was chosen for the next sets of experiments (Fig. S1a). The pH-sensitive characteristic relates to the surface protonation and deprotonation of N-CDs. The fluorescence intensity of the N-CDs was insensitive to NaCl concentration (Fig. S1b), guaranteeing the applicability of the N-CDs in biological labeling and environmental analysis. The effect of incubation time on the fluorescence intensity of the N-CDs – Co^{2+} system was shown in Fig. S1c. The fluorescence intensity was notably stable within 3 mins, so 3 mins was selected as the incubation time in the follow-up

experiments.

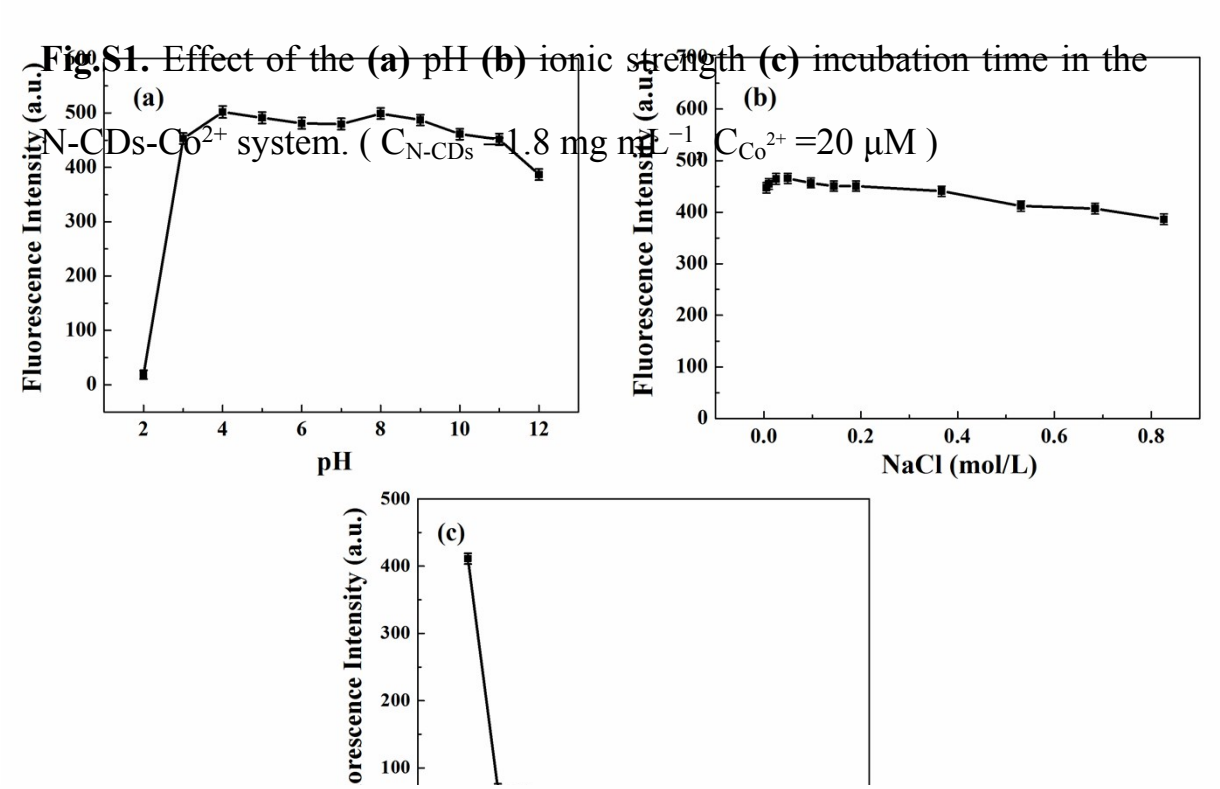

Fig.S1. Effect of the **(a)** pH **(b)** ionic strength **(c)** incubation time in the N-CDs-Co^{2+} system. ($C_{N\text{-}CDs}$ =1.8 mg mL^{-1}, $C_{Co^{2+}}$ =20 μM)

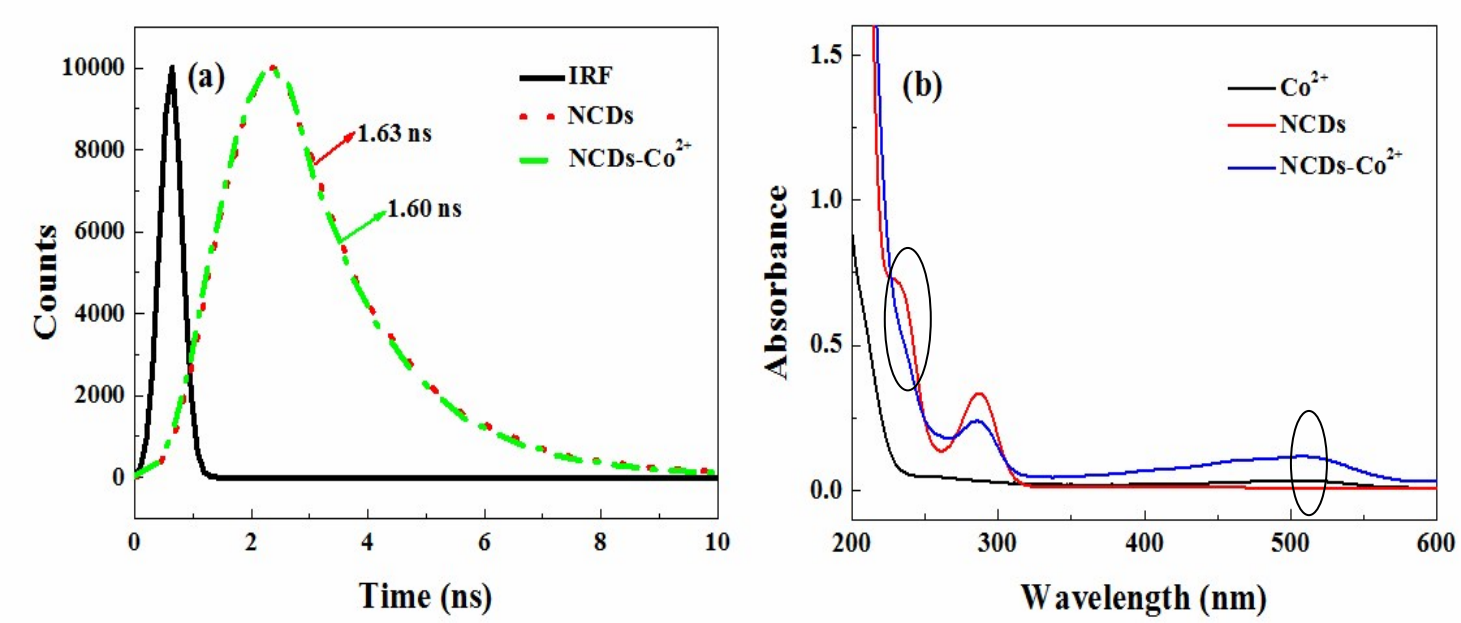

Fig.S2. (a) Fluorescence lifetime curves of the IRF and N-CDs in the absence/presence of Co^{2+}. **(b)** UV-vis absorption of the Co^{2+}, N-CDs and

N-CDs-Co^{2+}.