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

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Experimental

Materials

O-phenylenediamine, urea, Al2(SO4)3, CdSO4·8H2O, CoSO4·7H2O, Cr2(SO4)3·6H2O, FeSO4, FeCl3·6H2O, HgCl2, KCl, MgCl2·6H2O, MnSO4·H2O, Ni(NO3)2·6H2O, Pb(NO3)2, Zn(NO3)2·6H2O, CuSO4·5H2O, 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 purchased from guoda
drugstore. Tap water was obtained from ShanXi University. Zebras
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:

$$Q_C = \frac{Q_R \cdot I_C}{I_R} \cdot \frac{A_R}{A_C} \cdot \left(\frac{n_C}{n_R}\right)^2$$

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 MgSO₄, 1 mM CaCl₂, 0.15 mM KH₂PO₄, 0.05 mM Na₂HPO₄, 0.7 mM NaHCO₃, 5 – 10% methylene blue; pH 7.5) at 28 °C 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°C, 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
Fig.S1. Effect of the (a) pH (b) ionic strength (c) incubation time in the N-CDs-Co$^{2+}$ system. ($C_{\text{N-CDs}} = 1.8 \text{ mg mL}^{-1}$, $C_{\text{Co}^{2+}} = 20 \mu\text{M}$)

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\textsuperscript{2+}. 