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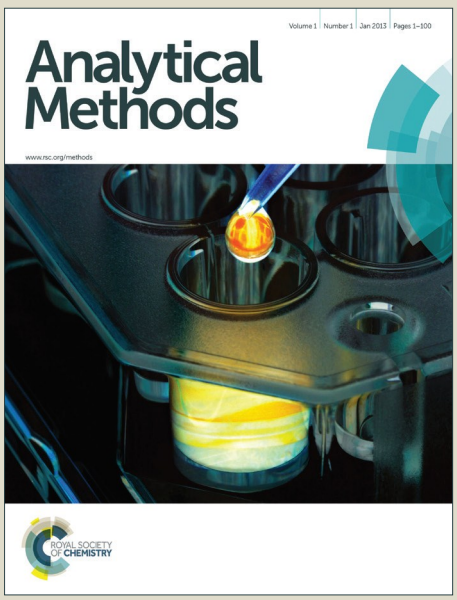

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A fluorescent probe based on N-doped carbon dots for highly sensitive detection of Hg^{2+} in aqueous solution

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Abstract

A facile and green hydrothermal method was developed for the preparation of highly luminescent nitrogen-doped carbon dots (NCDs) by using anhydrous citric acid and urea as carbon source and nitrogen source, respectively. The NCDs show good water-solubility and exhibit excitation-independent fluorescence behaviors at the excitation of 300-390 nm with a quantum yield of 42.5% at λ_{em} of 440 nm. Based on the fluorescence quenching strategy, the NCDs were successfully applied to the measurement of Hg^{2+} in tap and lake water samples with high sensitivity and excellent selectivity. The detection limit was 7.3 nmol L^{-1} (3σ, n = 9), indicating its potential applications to the detection of trace Hg^{2+} in water samples.

Introduction

Mercury (Hg) is a persistent pollutant with high toxicity that can be easily bio-accumulated in human body through food-chain, which has raised serious health concerns for decades.[1, 2] Therefore, the development of detection methods for trace Hg^{2+} in aqueous solution with high sensitivity and selectivity is of great necessity. The conventional analytical techniques, including cold-vapor atomic

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absorption spectrometry (CV-AAS),[3] cold-vapor atomic fluorescence spectrometry (CV-AFS),[4] and inductively coupled plasma mass spectrometry (ICP-MS)[5], have been extensively used for the measurement of Hg^{2+}. However, most of these methods require large-scale instruments and sophisticated sample preparation, which restricts their practical applications in the routine monitoring of Hg^{2+}.[6] Thus, it is still of a great challenge to develop a rapid and simple method for the detection of trace Hg^{2+} in aqueous solutions.

In recent years, fluorescent carbon dots (FCDs) have gained growing interests in bioimaging, biological labeling and sensing[7-9] due to the advantages of good water-solubility, low-toxicity and excellent optical properties. Since FCDs were first discovered in the purification procedures of single-walled carbon nanotubes in 2004,[10] a variety of preparation methods of FCDs have been developed, including laser ablation,[11] ultrasonic,[12] microwave-assisted,[13] electrochemical[14] and hydrothermal methods,[15] in which hydrothermal synthesis strategy is considered as a simple, direct and efficient way to obtain FCDs.[16]

Based on the fluorescence quenching strategy, FCDs have been used as biosensors for the detection of heavy metals.[18-21] For example, Dong et al.[22] described a novel sensing system of branched poly(ethylenimine) functioned carbon dots for Cu^{2+} detection with a detection limit of 6 nmol L^{-1}. Li et al.[18] showed the first use of carbon dots obtained from carbon soot by lighting a candle as a cheap, effective fluorescent sensing platform for Ag^{+} detection with high selectivity. Lu et al.[7] used pomelo peel as the carbon source to synthesize FCDs by hydrothermal method for the detection of Hg^{2+}, in which the sensing principle is presumably due to the formation of a stable non-fluorescent complex between FCDs and Hg^{2+}.[23]

Herein, a facile and green method was developed for the synthesis of highly luminescent

nitrogen-doped carbon dots (NCDs) by one-pot hydrothermal method. The NCDs were further used as a biosensor for the detection of Hg^{2+} in aqueous solution with high sensitivity.

Experimental

Reagents and materials

Anhydrous citric acid and urea were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). $HgCl_2$, $CdCl_2 \cdot 2.5H_2O$, $CuSO_4 \cdot 5H_2O$, $Pb(NO_3)_2$, $ZnCl_2$, $MnSO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$, $K_2Cr_2O_7$, $SrCl_2 \cdot 6H_2O$, $CaCl_2$, $FeCl_3 \cdot 6H_2O$ and quinine sulfate were purchased from Xilong Chemical Co., Ltd (Guangdong, China). The stock solution of Hg^{2+} (2.5 mmol L^{-1}) was prepared by dissolving $HgCl_2$ in ultra-pure water containing 1% (v/v) HCl. The standard solution of Hg^{2+} (1000 mg L^{-1}, GSB 04-1729-2004) was purchased from National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials (Beijing, China). All chemicals were of analytical grade, and were used without any further purification. The water used in all the experiments was purified with TKA Ultra (18.2 MΩ cm, Germany).

Apparatus

The UV absorption spectra were recorded with a UV-2100 spectrophotometer (Beijing LabTech, China). Fluorescence spectra were measured on a Varian Cary Eclipse fluorescence spectrophotometer (South East Chemicals & Instruments Ltd., Hong Kong, China). The Fourier transform infrared (FT-IR) spectra were measured on a Nicolet 380 FT-IR spectrometer (Thermo Scientific, US). Transmission electron microscopy (TEM) images were recorded with a JEM-2100 microscope (JEOL, Japan) operating at an accelerating voltage of 200 kV. X-ray photoelectron spectroscopic (XPS) measurement was performed with an Escalab 250Xi spectrometer (VG

Scientific, UK). An AFS-640 hydride generation atomic fluorescence spectrometer (HG-AFS) (Beijing Ruili Instrumental Co., China) was used for the measurement of Hg^{2+} in tap and lake water samples. The pH values were measured using a pH510 meter (EUTECH, Singapore).

Synthesis of NCDs

NCDs were synthesized according to a reported method with slight modification.[24] Briefly, 0.5 g of anhydrous citric acid and urea were dissolved in 10 mL of deionized water, and were transferred to a stainless-steel Teflon-lined vessel, followed by hydrothermal treatment at 160 °C for 4 h in an oven (DHG-9146A, Shanghai Jing Hong Laboratory Instrument Co., Ltd.). After the vessel cooled to room temperature naturally, the obtained black-green solution in vessel was precipitated with acetone thrice to provide the NCDs. After dried under vacuum, the as-prepared NCDs were re-dispersed in acetate buffer (10 mmol L^{-1}, pH 6.0) at a concentration of 45 g L^{-1} for further characterization and application.

Quantum yield measurement

The quantum yield (QY) of the synthesized NCDs was calculated using quinine sulfate as reference. Quinine sulfate (QY = 0.54 at 360 nm) was dissolved in H_2SO_4 (0.1 mol·L^{-1}, refractive index (n) = 1.33), while the NCDs were dissolved in acetate buffer solution (pH 6.0, n = 1.33). Their fluorescence spectra were both recorded at an excitation wavelength of 360 nm. To minimize the re-absorption effects, the absorbance of both solutions was limited below 0.1 at 360 nm.[25] The quantum yield was calculated using the following equation:

$$Q_c = Q_s \times \frac{F_c \times A_s \times n_c^2}{F_s \times A_c \times n_s^2}$$

Where Q is the quantum yield, F is the integrated emission intensity, n is the refractive index of the solvent, and A is the optical absorbance at 360 nm measured with a UV–Vis spectrophotometer. The subscript "s" and "c" refer to quinine sulfate with known QY value and NCDs, respectively.

Fluorescence detection of Hg^{2+}

In a typical assay, 1.5 mL of NCDs (1.2 μg mL^{-1}) in acetate buffer solutions (10 mmol·L^{-1}, pH 6.0) was mixed with 0.5 mL of Hg^{2+} at different concentrations in centrifugal tubes (2 mL). After incubation for 2 min at room temperature, the mixed solutions were recorded for fluorescence intensities at an excitation wavelength of 340 nm.

Tests of selectivity and interferences

The selectivity of NCDs was examined by mixing NCDs with different interference ions including Cd^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Cr^{6+}, Fe^{3+}, Sr^{2+}, Ca^{2+} and Zn^{2+}. The fluorescence intensities of NCDs in the absence (F_0) and presence (F) of the interference ions were measured. The F/F_0 ratios of the interference ions were compared with that of Hg^{2+} at the same metal concentrations (20.0 μmol L^{-1}). For the interference testes, the NCDs solutions were respectively mixed with each of the interference ions, followed by the addition of equal dosage of Hg^{2+} at 10.0 μmol L^{-1}. After incubation for 2 min at room temperature, the solutions were measured for the fluorescence intensities at λ_{em} of 440 nm (λ_{ex} = 340 nm).

Detection of Hg^{2+} in real samples

The practicality of NCDs-based probe for the detection of Hg^{2+} in tap water and lake water samples was respectively evaluated. The tap water was obtained from our laboratory and was analyzed

without any further pretreatment. Another two water samples collected from local lakes were filtered through 0.22 μm nylon membrane before analysis. Each of the water samples was mixed with NCDs for 2 min at room temperature. Then, the fluorescence quenching intensity at λ_{em} of 440 nm was measured. The recovery experiments were also carried out by spiking the water samples with Hg^{2+} standards at three levels.

Results and discussion

Synthesis of NCDs

The NCDs-based probe was prepared by a facile one-step hydrothermal method. Citric acid and urea were used as the carbon source and nitrogen source, respectively. Under the condition of high temperature and high pressure, a polymer-like material was formed between citric acid and urea through a condensation polymerization reaction, and was further carbonized to form NCDs.[26] For the optimization of the performance of NCDs, several synthesis parameters such as temperature, reaction time and the ratio between citric acid and urea were investigated. In a typical optimization, the amount of anhydrous citric acid and the solvent volume were kept a constant value, 0.5g and 10mL respectively. The other synthetic conditions varied as follows: (a) the amounts of urea (0.17-2.5 g); (b) reaction temperature (140-200 °C); (c) reaction time (3-6 h). The optimization results shown in Fig. S1 were described as follows: (a) a mass ratio of 1:1 between citric acid and urea, (b) the reaction temperature of 160 °C, (c) the reaction time of 4 hours.

Characterization

The as-prepared NCDs display clear green color and emit blue fluorescence under the illumination

of UV light at 365 nm. The fluorescent quantum yield is 42.5% using quinine sulfate (QY 54% in 0.1 mol L^{-1} H_2SO_4, λ_{ex} = 360 nm) as the reference. It can be clearly seen in Fig. 1A that the NCDs show two obvious absorption peaks at 235 nm and 340 nm. The absorption peak at 235 nm can be ascribed to the typical π-π^* transition of the aromatic sp^2 bond, while the absorption peak at 340 nm is due to the n-π^* transition of C=O or C-OH bond in the NCDs.[27, 28] The fluorescence spectra (Fig. 1B) indicate that the optimal excitation and emission wavelengths of NCDs were 340 nm and 440 nm, respectively. It should be noted that neither citric acid nor urea solution emits luminescence in visible region at λ_{ex} of 340 nm, revealing that the bright blue fluorescence originates from NCDs. The maximum emission wavelength (λ_{em}) of NCDs at 440 nm remains unchanged with the excitation wavelengths increasing from 300 to 390 nm, and the highest fluorescence intensity appears at λ_{ex} of 340 nm (Fig. 1C). The excitation-independent photoluminescence behavior might be attributed to the less surface defects and the narrow particle size distributions of NCDs.[29, 30]

Fig. 2B shows the FT-IR spectra of the as-prepared NCDs. The peak at 3430 cm^{-1} is attributed to the stretching vibrations of O–H and N–H, and the peak at 3170 cm^{-1} corresponds to the stretching vibration of C=C-H.[31] The peaks around 2920 and 1660 cm^{-1} are accordance with the vibrations of C-H and C=O bonds, respectively.[24, 32] Two characteristic peaks at 1600 cm^{-1} and 1400 cm^{-1} can be attributed to the asymmetric and symmetric stretching vibration of COO^-, respectively.[7] The results reveal that the NCDs are functioned with plentiful hydrophilic groups such as hydroxyl, carboxyl and amino groups, which endows the NCDs with favorable water solubility.

The morphologies of NCDs characterized by TEM are shown in Fig. 2B. The TEM images illustrate that the NCD particles are spherical and well dispersed without apparent aggregation with

an average diameter about 2.4 nm. The surface composition and elemental analysis of the NCDs were characterized by XPS. The peaks at 284.8, 400.2 and 531.5 eV in a full scan XPS spectrum (Fig. 3A) can be attributed to C1s, N1s, and O1s, respectively.[33] And the mass percentages of C, N and O of the NCDs are 51.25%, 14.5% and 34.25%, respectively. In the high-resolution region of C1s as shown in Fig. 3B, there are three peaks at 284.8, 286.8 and 288.4 eV which are attributed to C-C/C=C, C-N and C=O groups, respectively.[7, 13, 32, 34] The two peaks of the O1s spectrum (Fig. 3C) at 531.2 and 532.7 eV are assigned to the C=O and C-OH/C-O-C groups, respectively,[13, 19] while the spectrum of N1s (Fig. 3D) shows three peaks at 399.4, 400.3 and 401.5 eV, which are attributed to C-N-C, N–$(C)_3$– and N–H groups, respectively.[7, 13, 16] In conclusion, the FT-IR and XPS spectra confirm that the surfaces of the NCDs have been functionalized with multiple groups containing oxygen and nitrogen atoms.

Fluorescence stability of the NCDs

The fluorescence intensities of the NCDs solutions under various conditions (low pH, high ionic strengths and longtime illumination) were measured to investigate the stability. Result in Fig. 4A shows that the fluorescence intensity of the NCDs is pH-dependent, in which the fluorescence intensities increase with pH from 3.0 to 6.0, but keep unchanged above pH 6.0. Therefore, pH 6.0 was chosen for the subsequent experiments. Result in Fig. 4B shows that the fluorescence intensities of NCDs keep constant at NaCl concentrations from 0.2 to 0.6 mol L^{-1}, followed by slight decreases above 0.8 mol L^{-1}, indicating a high stability of the NCDs under high ionic strength environment. This behavior is favorable for the potential application in biological labeling and analytical detection. Moreover, the NCDs are found to present a high resistance of photobleaching

at continuous UV illumination (365 nm) as long as 1 h as shown in Fig. 4C, which may be a consequence of the chemical structure of the NCDs with no graphite lattices.[32] Result in Fig. 4D shows that the NCDs exhibit stable emission in that they only lose about 3% of fluorescence after storage at -4 °C for three weeks.

Selectivity of the NCDs probe for Hg^{2+} detection

Selectivity is an important parameter to evaluate the performance of NCDs as a fluorescent probe for Hg^{2+} detection. Therefore, the fluorescence intensities of NCDs were respectively analyzed in the presence of various metal ions including Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Cr^{6+}, Fe^{3+}, Sr^{2+}, Ca^{2+} and Zn^{2+} at the same concentrations. Results in Fig. 5A show that the addition of metal ions causes slight decrease of fluorescence intensities except for Hg^{2+} that induces strong fluorescence quenching of NCDs immediately. Meanwhile, the results of interference testes as shown in Fig. 5B indicate that the addition of Hg^{2+} induces obvious fluorescence quenching even in the presence of each interference metal ion, indicating that these metal ions do not interfere with the detection of Hg^{2+} except for Cr^{6+} and Fe^{3+}. With a large ionic radius and a special electronic layer structure, Hg^{2+} is prone to be polarized and deformed by the coordinating atoms of O and N on the surface of NCDs, thus forming stable NCDs-Hg^{2+} complexes through partial covalent bonds, which might be the main reason for the outstanding selectivity and specificity of NCDs to Hg^{2+}.[13] As a strong electron acceptor to the NCDs,[35] Hg^{2+} has stronger affinity to carboxylic, hydroxyl and amino groups on the surface of the NCDs than the other metal ions.[13] Furthermore, NCDs-Hg^{2+} complexes may facilitate charge transfer and restrain the radiative recombination of excitons, leading to the significant fluorescence quenching effects.[14]

Optimization of the Hg^{2+} detection conditions

Experimental conditions such as buffer compositions, NCDs concentrations and incubation time were optimized based on detection sensitivity and accuracy. Results in Fig. S2A show that the buffer compositions at pH 6.0 almost do not affect the initial fluorescence intensities of NCDs, but present obvious effects on the fluorescence quenching after the addition of Hg^{2+}. Therefore, acetate buffer (10 mmol L^{-1}) with pH 6.0 was chosen as the aqueous medium for Hg^{2+} detection.

NCDs at different concentrations present a sensitive measurement for Hg^{2+} by quenching effect. As shown in Fig. S2B, 1.2 μg mL^{-1} of NCDs is found to present the highest quenching efficiency ($F/F_0 = 0.81$) for 1 μmol L^{-1} of Hg^{2+}. The result shows that the fluorophore at very low concentration can't be effectively quenched by Hg^{2+}, similarly, for a given concentration of Hg^{2+} the quenching efficiency decreases at the high concentrations of fluorophore. It has been found that the lower concentration of fluorophore, the larger the change of F/F_0 value.[36] Therefore, 1.2 μg mL^{-1} of NCDs was chosen in the present study. The results in Fig. S2C show the dependence of incubation time on the fluorescence quenching. It costs 80 seconds only to reach quenching equilibrium, indicating that the quenching rate is fairly fast.

Linear range

To evaluate the sensitivity of this system, Hg^{2+} standard solutions with different concentrations were mixed with the NCDs under the optimized conditions. As shown in Fig. 6A, the fluorescence intensity of the NCDs is sensitive to the concentration of Hg^{2+}. Fig. 6B clearly shows the calibration curve of F/F_0 *versus* the concentrations of Hg^{2+} ions. The inset plot of Fig.6B indicates a good linear correlation ($R^2 = 0.9992$) can be obtained with the concentrations of Hg^{2+} ranging from 0.05

μmol L^{-1} to 5 μmol L^{-1}.

Hg^{2+} measurement in real samples

One tap water and two lake water samples were used for the Hg^{2+} measurement by the NCDs fluorescent probe together with HG-AFS to verify the accuracy and reliability of the analytical procedure. The results listed in Table 1 show that the analytical data of the NCDs fluorescent probe method is in accordance with the verification values of HG-AFS method. The detection limit of the NCDs fluorescent probe method is 7.3 nmol L^{-1} (3σ, n = 9), which is comparable to the previous reports (Table 2), especially much lower than that of the NCDs fluorescent probe for Hg^{2+} detection [38], and may be due to the different sources of carbon and nitrogen used and the different synthesis conditions compared with these previous NCDs preparation. In addition, it was found that humic acid with concentration ranging from 1-100 μmol L^{-1} in water does not interfere with the fluorescent quenching efficiency of Hg^{2+} as shown in Fig. S3. Because the detection limit is also lower than the maximum permitted value (10 nmol L^{-1}) of mercury in drinking water (Environmental Protection Agency of the United States),[13] the developed NCDs-based sensor presents a promising sensing platform for the detection of Hg^{2+} in freshwater including drinking water.

Conclusion

In summary, the highly luminescent N-doped carbon dots (NCDs) were synthesized by a facile one-step hydrothermal method with anhydrous citric acid and urea as carbon source and nitrogen source, respectively. The as-prepared NCDs, with a quantum yield of 42.5%, exhibit outstanding

selectivity and sensitivity of fluorescence quenching for Hg^{2+} and can be served as an effective fluorescent sensing probe for quantitative detection of trace Hg^{2+} in aqueous solutions with a detection limit of 7.3 nmol L^{-1}.

Acknowledgements

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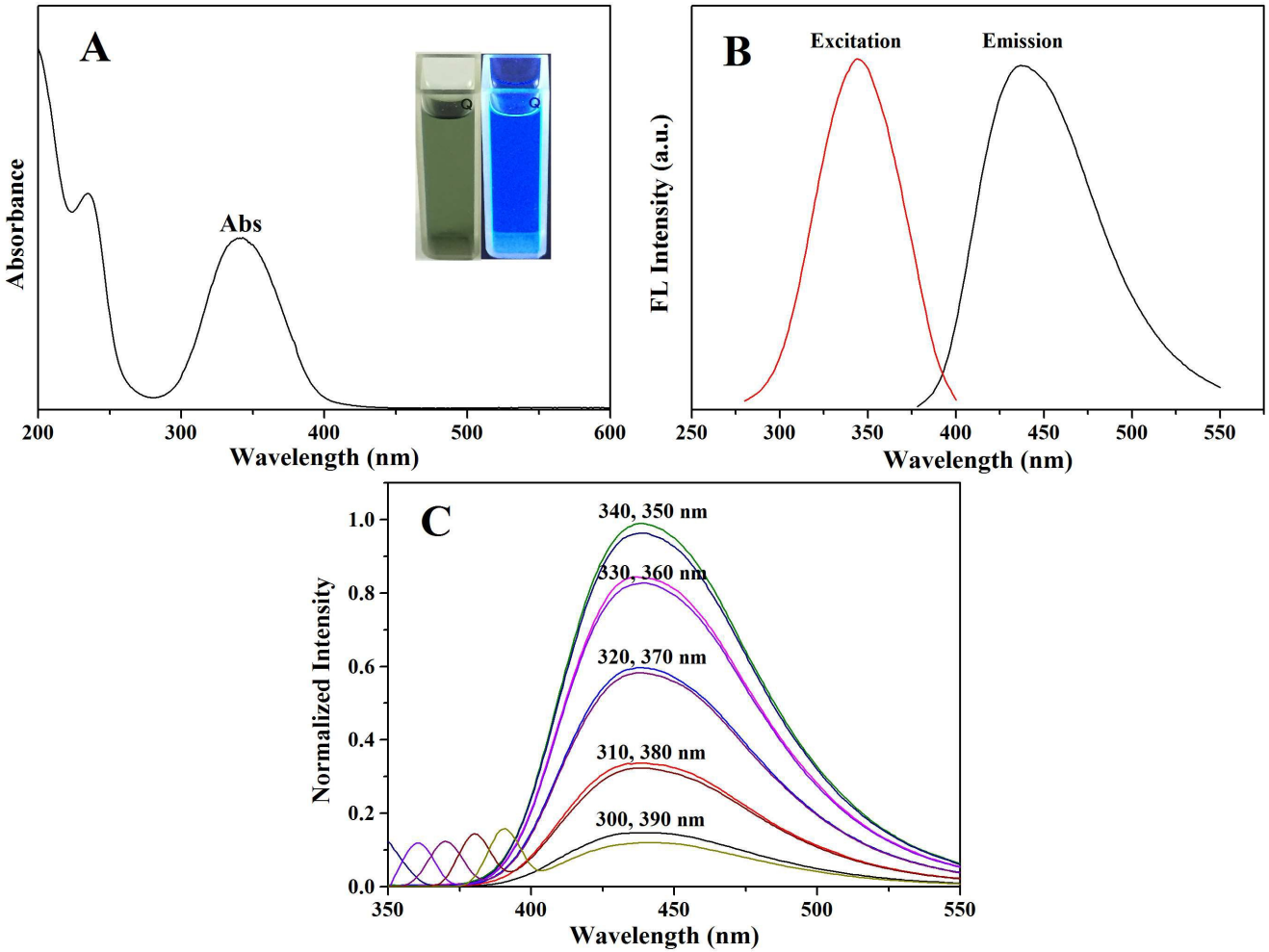


Fig. 1. (A) UV–vis absorption spectrum of the NCDs. Inset shows the colors of the NCD aqueous solution under visible light (left) and UV light (right). (B) Fluorescence excitation and emission spectra of the NCDs. (C) Emission spectra of the NCDs at different excitation wavelengths from 300 to 390 nm.

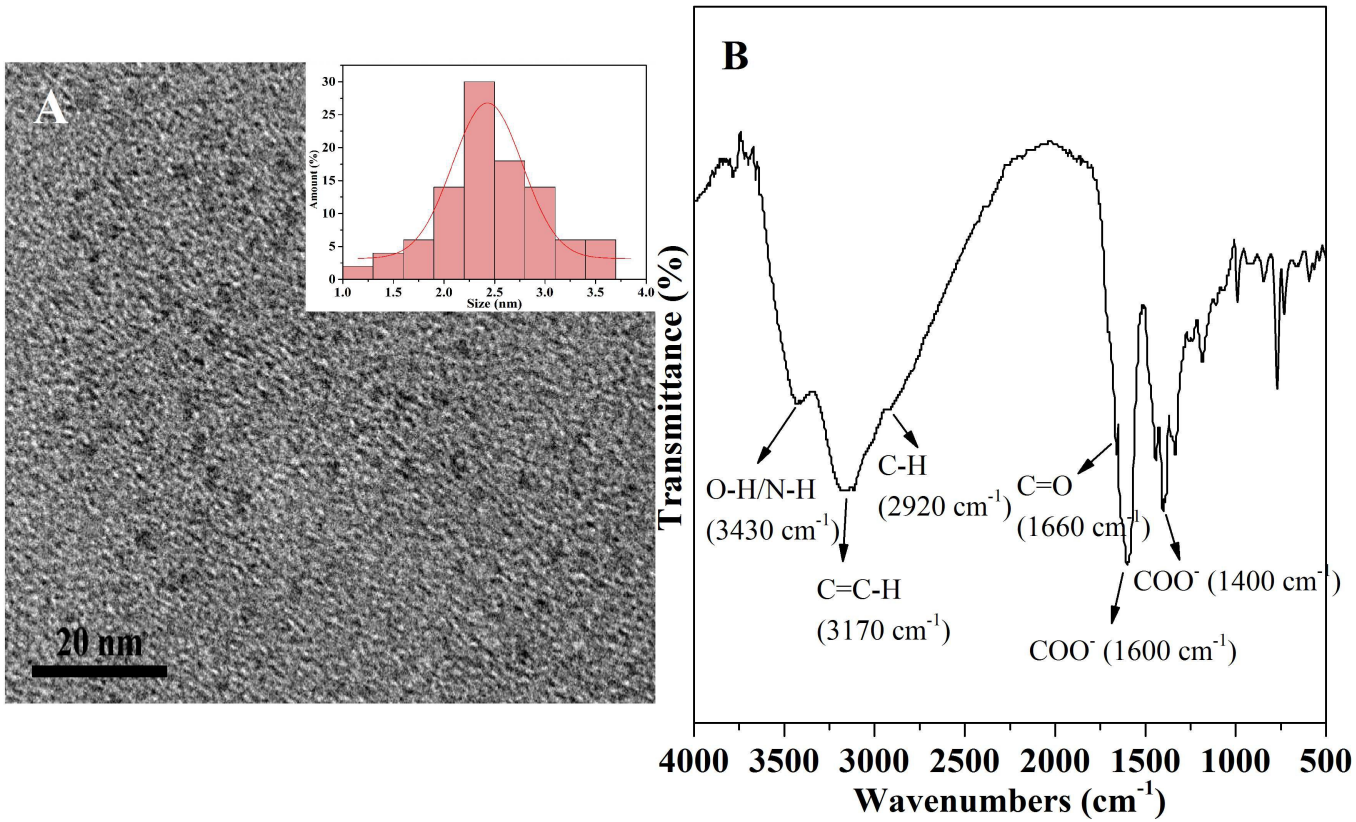


Fig. 2. (A) TEM image of the NCD particles. Inset shows the distribution of particle sizes. (B) FT-IR spectrum of the NCDs.

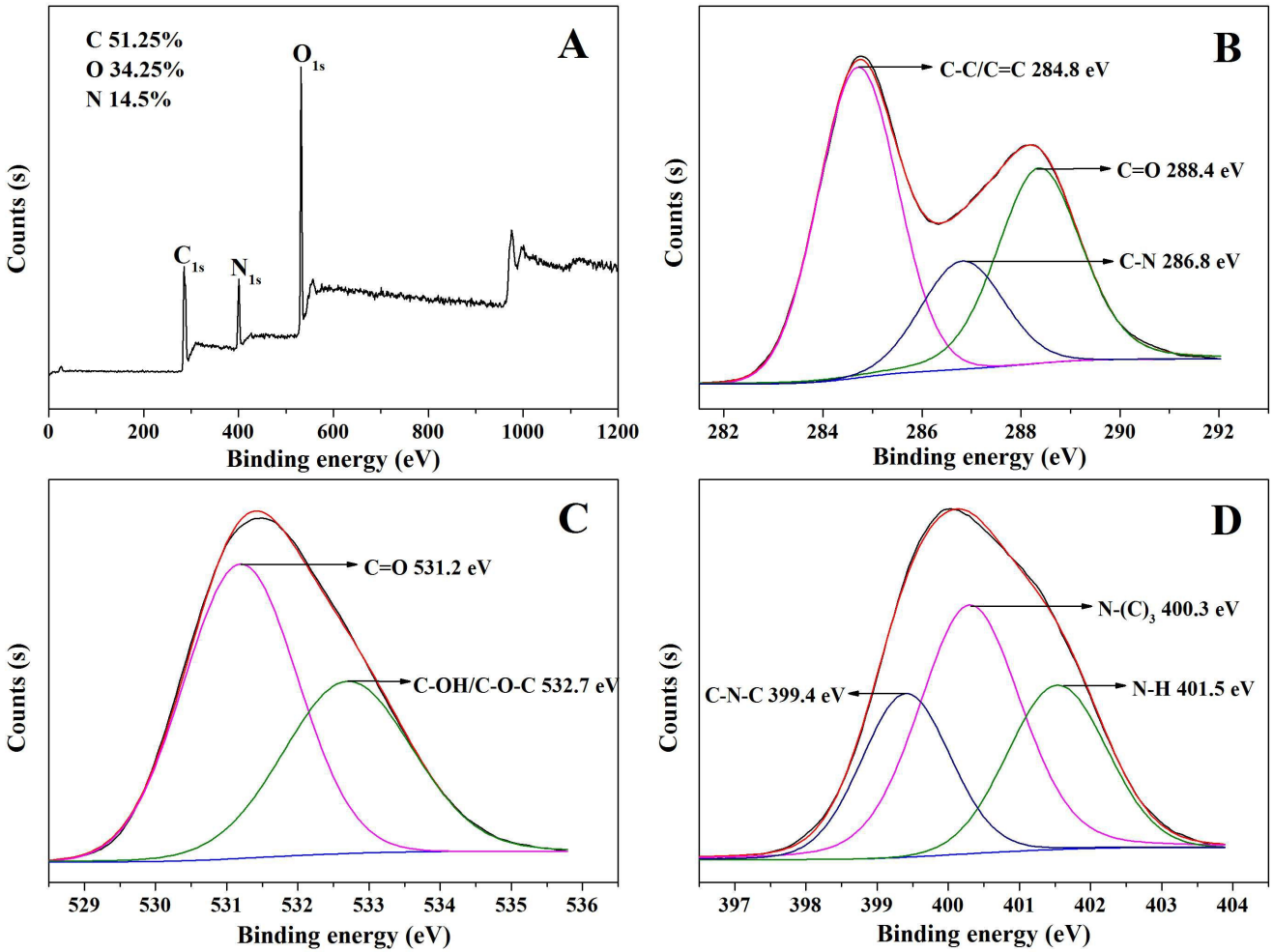


Fig. 3. (A) XPS spectra of the NCDs. (B-D) High-resolution XPS spectra of C1s (B), O1s (C) and N1s (D).

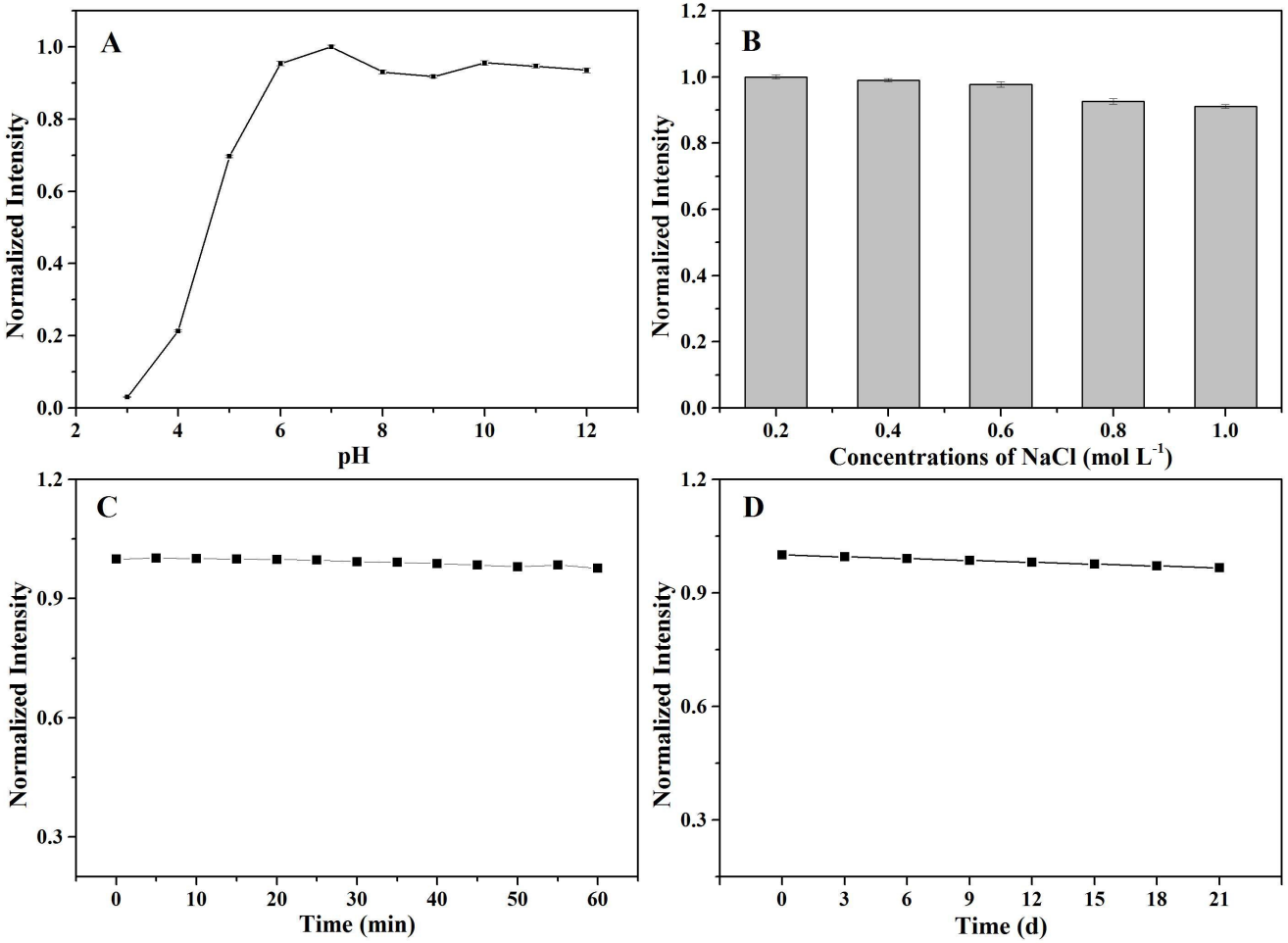


Fig. 4. (A) Normalized fluorescence intensities of NCDs at different pH. (B) Fluorescence response of NCDs in the presence of different concentrations of NaCl. (C) Normalized fluorescence intensities (440nm) of NCDs under the UV illumination of 365 nm at different times. (D) Normalized fluorescence intensities (440nm) of NCDs stored in a refrigerator up to three weeks.

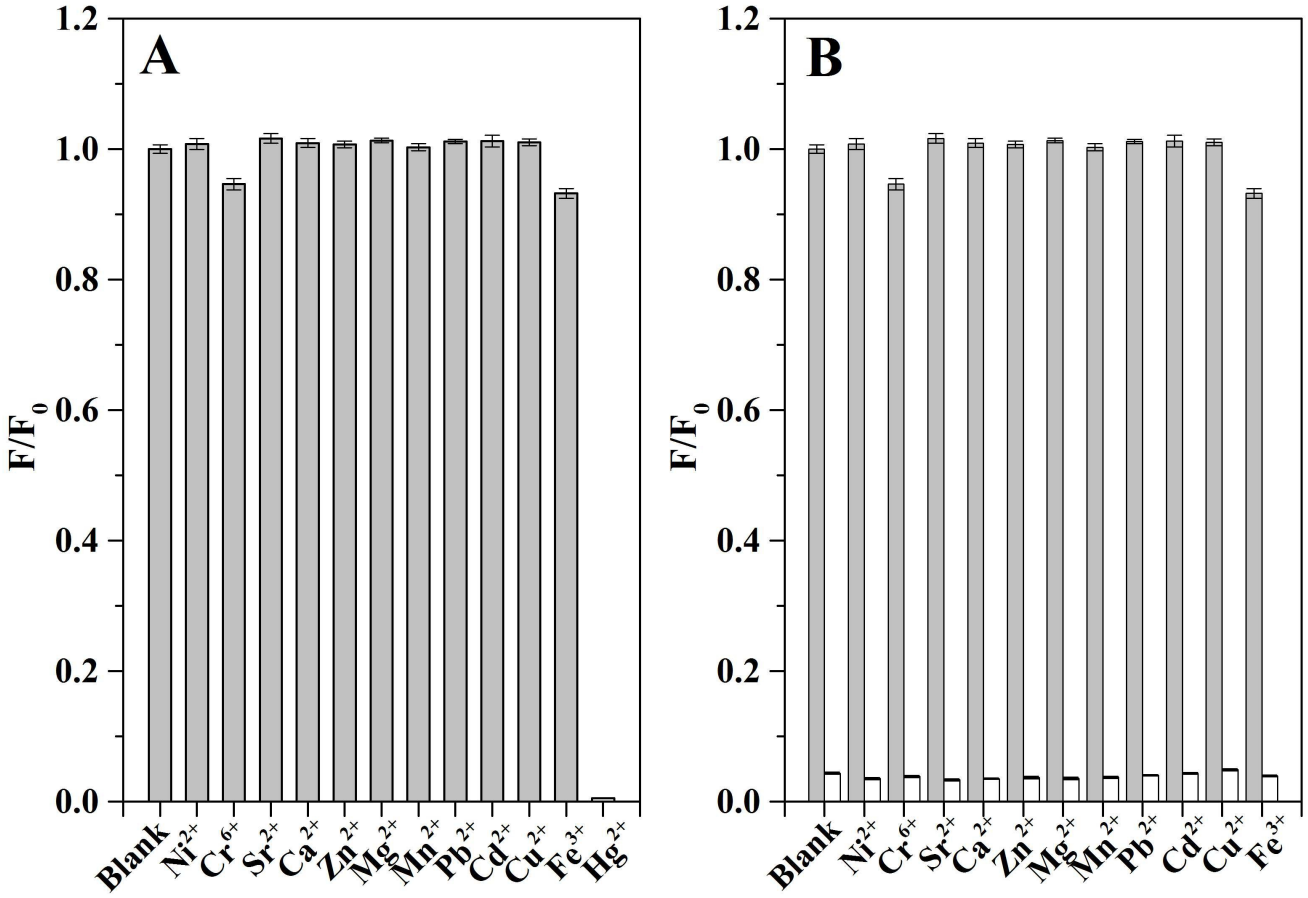


Fig. 5. (A) Fluorescence responses of NCDs to different metal ions under acetate buffer (10 mmol L^{-1}, pH 6.0).. The concentration of each metal ion is 20 μmol L^{-1}. F_0 and F correspond to the fluorescence intensities of NCDs at 440 nm excited at 340 nm in the absence and presence of metal ions, respectively. (B) The fluorescence intensities of M+FCDs (M represents any one of metal ions except for Hg^{2+}) solutions before (grey) and after (white) mixing with Hg^{2+} (10.0 μmol L^{-1}).

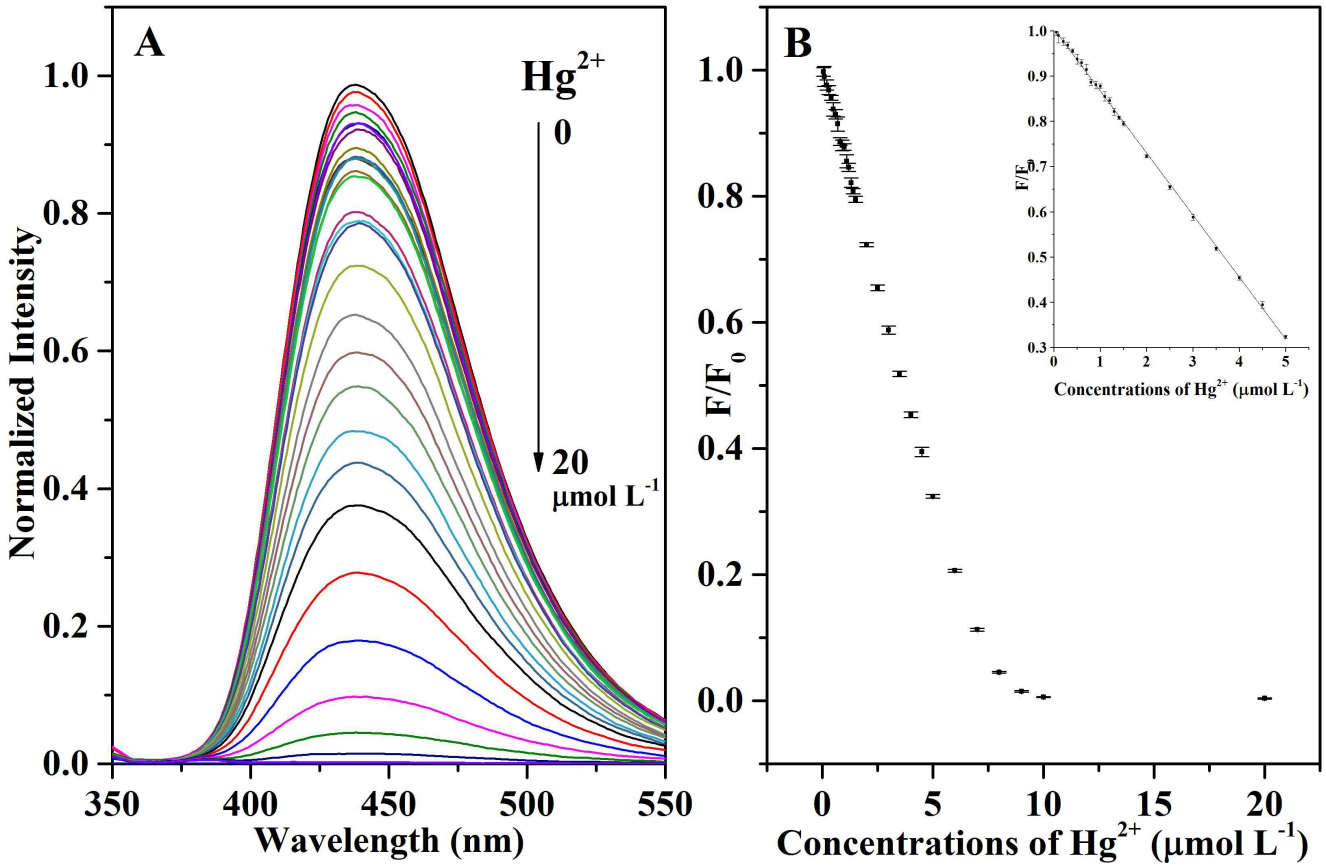


Fig. 6. (A) Emission spectra of NCDs mixed with Hg^{2+} at different concentrations (0 to 20 μmol L^{-1}) in acetate buffer (10 mmol L^{-1}, pH 6.0). (B) F/F_0 of NCDs (λ_{ex}=340 nm, λ_{em}=440 nm) mixed with Hg^{2+} versus the concentrations of Hg^{2+}. The inset plot shows the linear region of the curve (*n*=3).

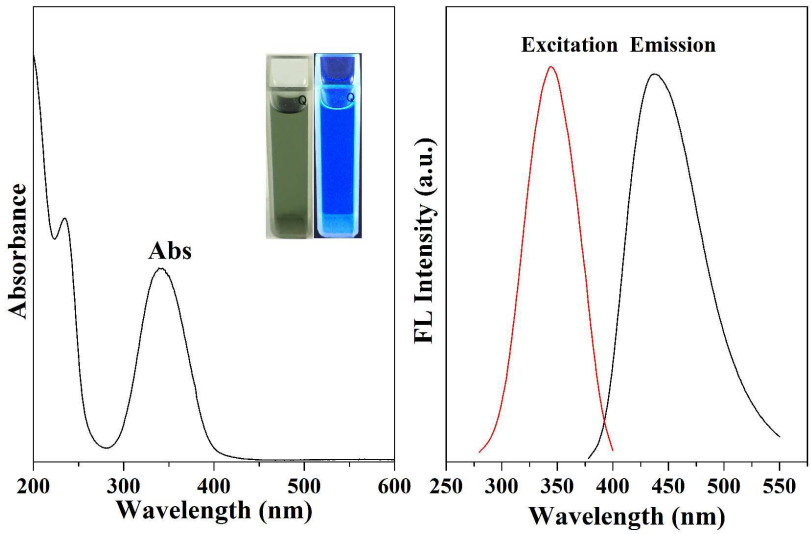
Table 1. Recovery tests of spiked Hg^{2+} in different water samples measured with NCDs and HG-AFS, respectively. (*n*=3)

Samples	Spiked Hg^{2+} ($\mu mol\ L^{-1}$)	NCDs		HG-AFS	
		Measured value ($\mu mol\ L^{-1}$)	Recovery (%)	Measured value ($\mu mol\ L^{-1}$)	Recovery (%)
Tap water	0.2	0.181 ± 0.007	90.4	0.196 ± 0.003	97.9%
	0.4	0.388 ± 0.008	97.1	0.399 ± 0.002	99.7%
	1.0	0.982 ± 0.007	98.2	1.003 ± 0.004	100.3%
Lake water 1	0.2	0.161 ± 0.007	81.7	0.196 ± 0.003	98.0
	0.4	0.332 ± 0.005	83.1	0.401 ± 0.002	100.3
	1.0	0.871 ± 0.004	87.1	1.012 ± 0.003	101.2
Lake water 2	0.2	0.168 ± 0.006	84.0	0.197 ± 0.002	98.2
	0.4	0.344 ± 0.004	86.1	0.402 ± 0.001	100.4
	1.0	0.886 ± 0.008	88.6	1.011 ± 0.003	101.1

Tap water was sampled from our laboratory. Lake water 1 and 2 were sampled from Jimei Lake and Jingxian Park respectively in Xiamen city, China.

Table 2. Detection limits and linear ranges of different fluorescent probes for Hg^{2+} detection.

Fluorescent probes	Linear range (μmol L^{-1})	Detection limit (nmol L^{-1})	Ref.
Carbon dots	0.0005–0.01	0.23	[7]
Carbon dots	0 – 3	4.2	[20]
Carbon dots	0 – 5	10	[37]
Nitrogen carbon dots	0 – 25	230	[38]
Fluorescent Ag clusters	0.01 – 5	10	[39]
Carbon dots-labeled ODN	0.005 – 0.2	2.6	[40]
N,S/Carbon dots	0 – 40	2×10^3	[41]
NCDs	0.05 – 5	7.3	This work



UV–vis absorption spectrum (left) and fluorescence excitation and emission spectra (right) of NCDs