Rapid determination of trace amount of chromium (VI) in water using carbon quantum dots as a fluorescence probe

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Carbon quantum dots (CQDs) were prepared using one step hydrothermal method using ammonium citrate and lactose as nitrogen and carbon sources. The prepared CQDs were characterized using XRD, TEM, XPS, FT-IR, UV-Vis, and fluorescence spectroscopy. The fluorescence quantum yield of the CQDs is 17.6 %. The CQD fluorescence sensor based on the quenching effect by Cr (VI) was successfully developed for quantitative determination of Cr (VI). Moreover, CQDs exhibited a sensitive and selective response to Cr^{6+} compared to other common ions in water (Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+}). The prepared CQD fluorescence probe demonstrates good linear detection performance in the concentration range of 0.10 ~ 0.60 mg/L for Cr (VI) ion. It is thus potentially feasible to detect industrial wastewater with discharge standard of less than 0.50 mg/L.

(Received November 12, 2021; Accepted February 16, 2022)

Keywords: CQDs, Fluorescent probe, Hexavalent chromium, Fluorescence analysis

1. Introduction

The photoinduced electron transfer properties of carbon quantum dots (CQDs) can be used for light energy conversion and nanoprobe detection of metal ions or organic molecules.Metal ions or organic molecules usually have a quenching effect on the fluorescence of CQDs, and the degree of fluorescence quenching has a linear relationship with the measured substance in the low concentration range, with low detection limit and high sensitivity. For example, N-doped carbon dots with photoluminescence (PL) quantum yield (QY) as high as 21% exhibited a sensitive and selective response to $Fe^{3+[1]}$.

The PLQY of CQDs is also affected by the size and microstructure, and the size-dependent PL property is a very important property of CQDs. CQDs of different sizes can be obtained by treatment with different carbon sources and nitric acid, which can be multicolor PL and emit light of different colors under excitation of different wavelengths ^[2]. Because of their excellent performance, CQDs may be applied to the field of heavy metal ion detection.

The CQDs prepared by different methods have different chemical structures and luminescence centers ^[3,4]. The optical properties of CQDs are affected by surface passivation or

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https://doi.org/10.15251/DJNB.2022.171.221

functionalization. There are a large number of carboxylic acid groups on the surface of CQDs, which can react with other organic, inorganic and biological groups to obtain special properties. The functional groups have important effect on the PL spectrum and intensity ^[5,6]. However, the PL mechanism of CQDs is still unclear, and the stability, uniformity and PL need to be improved. Therefore, it is of strong interest to develop low cost and efficient methods for synthesizing CQDs with controlled size and PL properties Functionalized CQDs have the potential for detecting heavy metals in water with high selectivity, sensitivity and reusability ^[7,8].

In this work, CQDs were prepared using one step hydrothermal method by adopting ammonium citrate and lactose as nitrogen and carbon sources, respectively. PL quenching of CQDs was used to detect Cr (VI). The results show that PL quenching is linearly proportional to the concentration of Cr (VI) in the range of interest for industrial wastewater detection (< 0.5 mg/L). This work demonstrates a versatile strategy for the synthesis of CQDs and their application for heavy metal ion detection (Scheme 1).



Scheme 1. Schematic diagram of fluorescence quenching detection principle.

2. Experimental

2.1. Materials

Ammonium citrate (AC), lactose, mannose, and the standard of hexavalent chromium stock solution were supplied by Sinopharm Chemical Reagent Co., Ltd. (Ourchem, China). Rhodamine B and metal ions (Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+}) were purchased from Aladdin Ltd. (Shanghai, China). All chemical reagents were analytical pure grade, and the experimental water employed was ultrapure water.

2.2. The preparation of CQDs

0.3603 g of lactose and 6.0805 g of ammonium citrate (molar ratio of 1:25) were added into a beaker with 30mL of deionized water, and then stirred for 30 min. The above solution was transferred to a 50 mL Teflon-lined autoclave and heated at 180 °C in an oven for 4 h. After the autoclave was naturally cooled to room temperature, the initial solution of CQDs was obtained by centrifugation (12000 r/min) for 1 h to remove large particles. The supernatant was taken put and further filtered by dialysis bag with the intercepted molecular mass (MD) of 3.5 kD, followed by a dialysis process for final solution of CQDs. The CQDs were stored at 4 °C in a dark bottle for further experiment.

2.3. Instruments and apparatus

The transmission electron microscopy (TEM) was performed using JEM-2000 high

resolution transmission electron microscopy (TEM) instrument (JEOL, Tokyo, Japan). A Rigaku D/Max-2400 X-ray diffractometer (XRD) was employed to check the phases of the obtained samples. Fourier transform infrared (FT-IR) spectra were obtained using the Nicolet IS50 FT-IR spectrometer (Thermo Scientific, MA, USA). Ultraviolet-visible (UV–Vis) absorption spectra were obtained using the UV-1601 spectrometer (Beifen-Ruili, Beijing, China). Fluorescence spectra were measured on a Hitachi F-7000 fluorophotometer (Japan).

2.4. Measurement of quantum yield

A Rhodamine B (quantum yield (QY) = 90%) ethanol solution was used as reference to determine the QY of CQDs as follows (Eq. (1)):

$$\Phi_{\rm x} = \Phi_{\rm s} (\mathbf{n}_{\rm x}/\mathbf{n}_{\rm s})^2 (\mathbf{A}_{\rm s}/\mathbf{A}_{\rm x}) (\mathbf{F}_{\rm x}/\mathbf{F}_{\rm s}) \tag{1}$$

where Φ is the QY, n is the solvent refractive index, A is the absorbance value (λ_{em} =430 nm), F is the integral area of the fluorescence peak, and subscripts x and s represent CQDs and Rhodamine B, respectively.

2.5. Fluorescence detection of Cr(VI)

At room temperature (25 °C), 1.0 mL CQDs solution was mixed with phosphate buffered solution containing Cr (VI) at various concentrations from 0.10-0.60 mg/L. The buffer solution was used to adjust the pH of the mixture and the sensitivity of CQDs toward Cr^{6+} was evaluated at pH 6.5. The final volume of the above mixture was 10.0 mL. After incubation for 30 min, its fluorescence spectra were recorded using an excitation wavelength of 330 nm. In the interference study, the fluorescence intensity of the CQDs in the presence of various metal ions (Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+}) was measured using a similar method.

2.6. Detection in real water samples

The real water samples with pretreatment from a chemical plant were selected to evaluate the effect of the CQDs method for detecting Cr(VI). The pretreatment process was as follows: first, centrifugation was performed at 8500 rpm for 15 min; second, the water sample as filtered three times using a 0.22 μ m microporous filtration membrane; third, the pH of the solution was adjusted to 6.5. Subsequently, the water sample were spiked with Cr(VI) standard stock solutions of various concentrations, and the aforementioned method was used for detection and analysis.

3. Results and discussion

3.1. Structural characterization of CQDs

The corresponding XRD pattern in Fig. 1 shows a broad diffraction peak centered at $2\theta=25.0^{\circ}$, suggesting the (002) plane of graphitic carbon, which agrees with the following Raman results described ^[9].



Fig. 1. The XRD pattern of CQDs.



Fig. 2. CQDs: (a) XPS full spectrum; (b) high-resolution C1s peaks; (c) high- resolution N1s peaks; (d) high-resolution O1s peaks.

To gain insight into the elemental states and the surface functional groups of the CQDs, XPS measurements were conducted. Fig. 2a shows that the XPS survey spectrum of the CQDs contained three obvious peaks at 284.8, 399.8, and 531.8 eV corresponding to C1s, N1s and O1s, respectively ^[10-13]. The C1s (284.8 eV) spectrum (Fig. 2b) was deconvoluted into four peaks at 284.5 eV (C=C), 285.3 eV (C-C), 287.8eV(C-O) and 288.5 eV (C=O). Thus, numerous oxygen-containing groups existed on the surface of the CQDs. As shown in Fig. 2c, the XPS spectrum has three deconvolution N1s peaks at 399.5, 400.6, and 401.6 eV attributed to C=N-H, N–H and C–N, respectively. The O1s (531.8 eV) spectrum (Fig. 2d) of the CQDs was

deconvoluted into three peaks at 531.0, 531.7 and 532.6 eV, which were assigned to C=O, H-O and C–O groups, respectively.



Fig. 3. (a) FTIR spectrum and (b) Raman spectrum of CQDs measured with 532 nm excitation.

The FTIR spectrum of the CQDs is shown in Fig. 3a. The broad peak around 3182 cm⁻¹ is attributed to the stretching vibration peaks of O-H and N-H bands ^[1]. The peaks at 1579 cm⁻¹ and 1402 cm⁻¹ are ascribed to C=C stretching mode and -COOH bond ^[14,15]. Other weak peaks were viewed at 775 cm⁻¹ and 638 cm⁻¹ are collected with the N-H bond and O-H band ^[16]. The results showed that there were $-NH_2$ groups and -OH groups on the surface of carbon quantum dots, which had good hydrophilicity and was favorable for binding with metal ions.

Raman spectrum reveals two different peaks of CQDs that are correlated with a disordered D-band and crystalline G-band centered at 1367 and 1584 cm⁻¹, respectively (Fig. 3b). The D-band is assigned to the sp³-hybridized carbon atom vibrations with dangling bonds, reflecting sheet defects ^[17]. The G-band corresponds to the E_{2g} mode of graphite, correlated with the in-plane sp²-hybridized carbon atom vibrations in the graphitic sheet. The intensity ratio (I_D/I_G) of the D-band to G-band was measured and shown to be 0.76, indicating that CQDs have a graphitic structure with enough structural defects ^[18].



Fig. 4. TEM images of CQDs.

The morphology of the CQDs was characterized using TEM, and the results are shown in Fig. 4. The CQDs particles were uniform with a particle size of 4~6 nm and an obvious lattice

fringes of about 0.21 nm, which corresponded to the (002) crystal plane of graphitic carbon (sp^2 hybrid carbon)^[19].

3.2. Optical properties of L-CQDs

The optical properties of CQDs were systematically investigated by UV-Vis absorption and fluorescence emission spectra. There appeared two peaks at 240 and 330 nm in UV–Vis absorption spectrum of CQDs (Fig. 5a), which are attributed to the π - π * energy transition of conjugated C=C bonds and n- π * energy transition of C=O bonds, respectively ^[20]. The prepared CQDs are colorless solutions under ambient light and emit a strong blue fluorescence under irradiation with 330 nm UV light (Fig. 5b). The fluorescence spectra showed the optimal excitation and emission wavelengths of CQDs at 330 and 440 nm, respectively. The PLQY of CQDs was calculated to be about 17.6 % according to the above mentioned equation. These findings confirm that CQDs have good optical properties based on UV-Vis and PL characteristics.



Fig. 5. (a) UV-Vis absorption spectrum of CQDs solution; (b) Fluorescence emission spectra of CQDs with 330 nm excitation.

3.3 Optimization of conditions

For the practical utility, the influence of pH, reaction time and the dosage of CQDs on the system were explored. As shown in Fig. 6a, the FL intensity reached the maximum value when the pH value was 6.5 in the CQDs system. In Fig. 6b, it was observed that the FL intensity showed a nearly constant when the reaction time was 30 min. The concentration of CQDs solution after dilution was 1.8~18.72 g•mL⁻¹ (measured in carbon), the fluorescence quenching degree of the system is obvious, which can be detected by fluorescence photometer. Therefore, the reasonable concentration of CQDs solution is 1.8~18.72 g•mL⁻¹(in carbon), pH 6.5 and 30 min of reaction time were selected as optimal reaction conditions.



Fig. 6. (a) The influence of pH on PL intensity; (b) The PL quenching efficiency of CQDs solution under different reaction time.

3.4. Fluorescence detection of Cr⁶⁺

3.4.1 Linear detection range of CQDs

In the system with hexavalent chromium ions of 0.10-0.60 mg/L, the quenching degree was increased with the increase of chromium ions concentration, showing a linear trend (inset in Fig. 7). When the chromium ion concentration is more than 0.60 mg/L, quenching degree is no longer a linear increase and the increase is small, which suggests that the quenching degree of CQDs can only be linear with a certain amount of chromium ions.



Fig. 7. Fluorescence emission of CQDs in the presence of different concentrations of Cr (VI).

The test results of the method of the invention are compared with those of the standard method as shown in Table 1.

Fluorescence quen	Spectrophotometry				
Calculated chromium	Average value	Standard	Chromium	effluent	
concentration (mg/L)	(mg/L)	deviation	concentration	concentration	
		(mg/L)	(mg/L)	(mg/L)	
0.0901, 0.0987, 0.1124,	0.1043	0.0127	0.1013	≤0.5	
0.0947, 0.1247, 0.1049					

Table 1. Comparison of this test method with the standard method.

From Table 1, CQDs fluorescence quenching method was detected in the linear range with a standard deviation of 0.0127 mg/L and a detection limit of 0.0399 mg/L. The average results of multiple parallel samples are close to the standard method, which can be used as a reference for rapid detection.

3.4.2. Interference experiment

On account of the complexity and diversity of actual ion environment, the interfering effects of various cations on the fluorescence quenching processes were investigated. Therefore, Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+} commonly found in water were used as interference ions to investigate the effect of CQDs on Cr^{6+} quenching, with shown in Fig. 8. The results show that only Cr^{6+} can quench CQDs, and the above common ions have little effect on the quenching. In the linear detection method, when the concentration of trivalent chromium ion (Cr^{3+}) is greater than 0.05 mg/L, Cr^{3+} has a significant effect on the linear detection of Cr^{6+} . Cr^{3+} in the solution will reduce the fluorescence intensity of quenching reaction by about 10%~15% compared with the control.



Fig. 8. Fluorescence quenching effect of different metal ions towards CQDs.

3.4.3. Real sample assay

1.000 L waste water from a chemical plant was taken, and the large particles were removed by centrifuge at high speed, filtered by dialysis filter membrane, heated and concentrated

to 100 mL. The subsequent steps were the same as the linear experimental method, and Cr^{6+} concentration in the water sample was determined. 1.00 mL of the CQDs mother solution was mixed with 1 mL of water sample solution, and then reacted in phosphoric acid buffer solution (pH 6.5) for 30 min to detect the fluorescence intensity. The final volume of the reaction solution was 10 mL. At the same time, the test method was added to the standard test, and Cr (VI) was determined by spectrophotometry, and the results were compared with those determined by the quenching test method, as shown in Table 2.

FL	Standard	Estimated	RSD(n=5)/%	Standard	Determinat	Recovery	Spectro
intensity	deviation	value		added	ion of total	%	photom
	(mg/L)	(mg/L)		(mg/L)	(mg/L)		etry
							(mg/L)
153.67	0.0115	0.2359	4.87	0.1005	0.3402	101.1	0.2231

Table 2. Determination of Cr (VI) content in water samples.

3.4.4. Fluorescence quenching mechanism

The mechanism of action may be that the surface of CQDs is rich in OH, C=O, C-N, N-H and other groups, which can bind to chromium ions, so that the luminescence unit is closed. The UV spectrum analysis before and after the quenching (Fig. 9) also confirmed that the quenching reaction of CQDs on chromium ion was influenced by the emitting unit, while the excitation unit was not affected and did not change.



Fig. 9. UV spectra of CQDs before and after the quenching.

A certain amount of CQDs can only quench a certain amount of chromium ions, which may be related to the binding performance of the surface active groups of CQDs with chromium ions. When chromium ion is not added, the CQDs absorb the energy of incident light, and the electrons in the ground state transition to the excited state. When the electrons in the excited state are returned to the ground state, the electron and hole recombination is formed to produce fluorescence. After adding chromium ions, fluorescence quenching can be achieved by combining with groups on the surface of CQDs.

4. Conclusion

In summary, CQD fluorescence sensor for detection of Cr (VI) was successfully developed. The CQDs were synthesized through a facile, hydrothermal method based on ammonium citrate and lactose. The maximum emission wavelength of CQDs was observed at 440 nm, under an excitation wavelength of 330 nm. In addition, CQDs exhibited a sensitive and selective response to Cr^{6+} compared to common ions in water (Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} and Mg^{2+}). The prepared CQDs fluorescence probe demonstrates good performance in the concentration range of 0.10 ~ 0.60 mg/L for Cr (VI) ion. It should be able to detect industrial wastewater with discharge standard of less than 0.50 mg/L.

Acknowledgements

This work was financially supported by Basic Public Welfare Research Program of Zhejiang Province (No. LGC21B050010) and Jiaxing Public Welfare Projects (No. 2020AD10021). No potential competing interest was reported by the authors.

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