Highly sensitive fluorescent probe for Cr^{3+} detection using carbon quantum dots

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Abstract. Carbon quantum dots (CQDs) had been widely applied in the metal ions detection area due to their excellent optical properties and low toxicity. Herein, a series of CQDs with different sizes were prepared by a facile but effective hydrogen peroxide (H_2O_2) oxidative method using activated carbon as carbon source. Experimental results showed that the concentration of H_2O_2 aqueous solution had critical effect on the sizes and surface structure of CQDs. When H_2O_2 aqueous solution was 20 wt.%, the diameter of CQDs was 3.6 nm, and the fluorescent probe exhibited a good linear response to Cr^{3+} in the concentration range from 0.2 to 55 μM with a detection limit of 0.2 μM. Through systematically analyzing FTIR, XPS and the optical properties of CQDs, the high sensitivity of CQDs for detecting Cr^{3+} was attributed to the synergistic effect of the surface oxygen-containing functional groups and size of CQDs.

Introduction

Chromium is one of the major water pollutants as a sequence of its widely used in the fields of metallurgy, chemical industry, electroplating, and high precision devices.[1] Trivalent chromium (Cr^{3+}), which is the oxidation state stable enough to exist in the environment, is poisonous and even carcinogenic for biological tissues because that Cr^{3+} can bind to DNA and destroy cellular structure of biology.[2] The World Health Organization (WHO) permits that the range of safe and adequate intakes for Cr^{3+} is 1-4 μmol per day[1] and the allowed concentration of Cr^{3+} in drinking water is 1 μM. Therefore, the trace detection of Cr^{3+} in environmental and biological samples is of great importance.

Spectrofluorimetry is an attractive method to the trace detection of Cr^{3+} ions considering its efficiency, easy manipulation, and cost-effectiveness.[1, 3] Based on the affinity of analytes, fluorescence-based probes, which are the key material for the detection of metal ions, can be reasonably designed for the detection of Cr^{3+} leading to signal changes of optics.[4] A gamut of fluorescence-base d probes has been used, including metal quantum dots (QDs), organic dyes, and fluorescent molecular probes. For the typical metal QDs, cadmium-based QDs (CdTe)'s toxicity has attracted increasing concern which limits their wide applications in the field of biology.[5, 6] The organic dyes and fluorescent molecular probes can be properly designed for determination of different targets based on their inherent structural properties. For instance, Saluja and co-workers designed benzimidazole-based fluorescent sensors for detecting Cr^{3+}, the resultant complexes of sensors and Cr^{3+} recognized oxyanions HSO_4^- bound to the metal center and done decomplexation with F^- occurred cation displacement.[7] However, the raw materials of organic dyes and fluorescent molecular probes are usually expensive and their preparation processes are often complicated, which limit their further development.[1, 7] Therefore, developing a novel fluorescent probe with extraordinary optical properties, low toxicity and facile preparation process has become increasingly significant and urgent.

Carbon quantum dots (CQDs), a new class of environmental friendly nanomaterial, had attracted great interest in the ions sensing field due to their good chemical stability, low toxicity, and good photostability.[8, 9] For instance, Liu *et al.* reported that the fluorescence (FL) of CQDs could be quenched by Cr^{3+} through combining with hydroxyl groups on the surface of CQDs, of which the detection limit of Cr^{3+} was 0.06 μM.[10] Savita *et al.* proposed a synthesis route to produce fluorescent CQDs with easily-available biomass such as wheat, rice, pearl millet, and sorghum as carbon materials, which were applied to detect Cr^{3+} and the detection limits of Cr^{3+} were up to 0.6, 1.2, 0.8, and 0.7 μM, respectively.[11] However, CQDs as fluorescent probes for detection of Cr^{3+} still need to be further improved in terms of selectivity and sensitivity. Furthermore, the quenching mechanism of CQDs for the detection of Cr^{3+} also should be systematically studied.

Activated carbon (AC), regarding as a common amorphous carbon, contained a large amount of graphite, which was most likely small crystal graphite pieces.[12] AC with long range disordered structure was therefore easily etched into small-sized CQDs by oxidation treatment. Additionally, the inexpensive, commercial available, and easy to obtain for AC also made it as an ideal carbon source for the preparation of CQDs. Herein, using AC as carbon source, a series of yellowish-green fluorescent CQDs with different sizes were prepared by a facile H_2O_2 oxidation method. Through simply controlling the concentration of H_2O_2 aqueous solution in chemical oxidative process, the size and the surface structure of the CQDs were controllably modified. The FL spectra show that the bright yellowish-green FL of the obtained CQDs can be only quenched in the presence of Cr^{3+}. The FL quenching effect was highly dependent on the size of the obtained CQDs, and the 3.6 nm CQDs showed the best detection performance with a detection limit of 0.2 μM. Furthermore, the obtained CQDs could be applied for detection of Cr^{3+} in real water samples.

Experimental

Preparation of fluorescent CQDs: The CQDs were prepared by a facile green method of H_2O_2 chemical oxidation. Briefly, 1.0 g AC was distributed into 50 ml H_2O_2 aqueous solution (20 wt.%) under ultrasonic stirring to form a homogeneous mixture. Then, the mixture was kept magnetic stirring at 100 ^oC for 12 h. After cooled to ambient temperature spontaneously, the product was filtered and centrifuged to remove the dark insoluble substances and large dots, and a brown aqueous solution containing CQDs was obtained, namely CQDs-4 hereafter. For comparison, CQDs with different sizes were prepared by the same method with H_2O_2 aqueous solution concentration of 4 wt.%, 10 wt.%, 15 wt.%, and 21 wt.%, which was labeled as CQDs-1, CQDs-2, CQDs-3, and CQDs-5, respectively.

Apparatus and characterization: The morphology and structure of the CQDs were analyzed by Tecnai 20 transmission electron microscope (TEM) at an acceleration voltage of 200 kV. Dynamic light scattering (DLS) of the CQDs was measured on a Zeta sizer Nano ZS analyzer. X-ray photoelectron spectra (XPS) were characterized by a ESCALAB250 spectrometer using Mg-Kα radiation at 200 W. Fourier transform infrared spectra (FTIR) were characterized with Nicolet iN10 MX & iS10 spectrometer using the KBr pellet technique. UV-visible absorption spectra (UV-vis) of CQDs were conducted on a LAMBDA 950 spectrophotometer. The FL spectra were recorded by a Hitachi F-7000 spectrofluorometer equipped with a 150 W xenon lamp as the excitation source.

Results and discussion

Structural characterization of CQDs

DLS and TEM were used to characterize the morphology, microstructure and size distribution of the CQDs samples. As shown in Fig. 1a, the five CQDs samples showed good dispersion, uniform particles size according to the DLS analysis. With the concentration of H_2O_2 aqueous solution increased from 4 wt.% to 21 wt.%, the sizes of the five CQDs samples decreased from 15.5 to 1 nm, suggesting that a series of CQDs with different sizes were prepared successfully by simple controlling the concentration of H_2O_2 aqueous solution in the reaction process. The representative TEM image of CQDs-4 further showed that the monodispersed nanoparticles were spherical (Fig. 1b). The corresponding size distribution histogram obtained by statistical analysis of the TEM image displayed that average diameter of the CQDs-4 was 2.4 ± 0.4 nm, which was little smaller than DLS result (3.6

nm). This may because that the size tested by DLS measurement was the average hydrodynamic diameter. To further identify the microstructure of the obtained CQDs, the HRTEM was performed on CQDs-4. The crystalline structures with lattice spacing of 0.24 nm can be observed (Fig. 1c), which can be corresponding with the (1120) lattice planes of sp^2 graphitic carbon.[13] The above data indicated that the facile green route of H_2O_2 oxidative method was efficient for the fabrication of CQDs. This preparation method did not require additional surface modification, no doping and purification process, which made it more facile and useful for practical application.

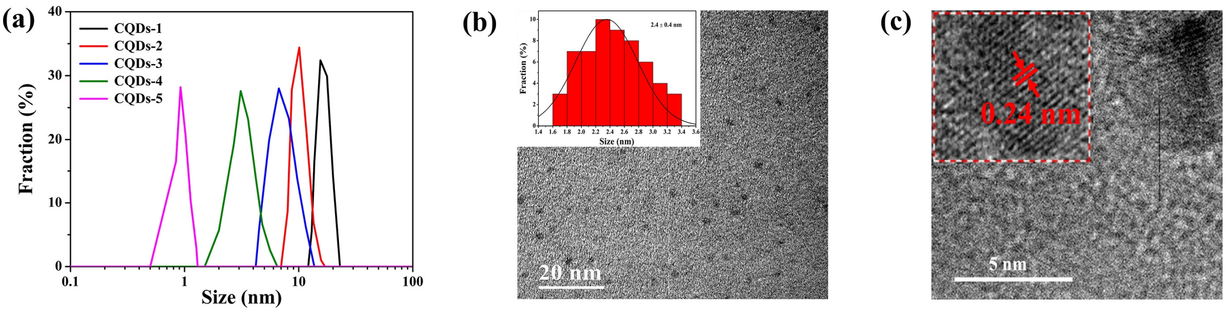

Fig. 1 (a) Size distributions of the five CQDs samples measured by DLS. (b) TEM image of CQDs-4 (inset: size distribution histogram of CQDs-4). (c) HRTEM image of CQDs-4.

The surface structure of the obtained CQDs had critical effect during their practical application. FTIR spectra were employed first to identify the surface functional groups of AC and the CQDs samples. The CQDs-4 displays mainly three new characteristic absorption bands at 3262 cm^{-1}, 1735 cm^{-1}, and 1450 cm^{-1} compared with AC (Fig. 2a), which can be indexed the stretching vibration of O-H, the stretching vibration of C=O, and the stretching vibrations of C-O, respectively. It indicated that large quantities of C=O and C-O were formed on the CQDs surfaces, which may be arising from the C=C and C-C bonds being broken down by •OH produced from H_2O_2.[14] XPS measurements were performed to further explore the chemical composition and element bonding configurations of CQDs-4. Its high-resolution C1s spectrum (Fig. 2b) was fitted by five peaks as C-C (284.6 eV), C=C (283.3 eV), C-O (285.6 eV), C=O (288.2 eV), and C-O-C (287.4 eV) were calculated as 28.4, 35.5, 14.1, 8.7, and 13.3 atom %, respectively.[15] The high-resolution O1s spectrum (Fig. 2c) can be deconvoluted into three peaks as C=O (531.4 eV), C-O-C (532.4 eV), and C-O (533.9 eV) with the corresponding atomic contents of 33.6%, 34.6%, and 31.8%, respectively[16]. It confirmed that oxygen-rich functional groups existed on the surface of CQDs. Oxygen-rich functional groups were beneficial to improve the hydrophilicity of the CQDs in aqueous solution, which may account for a great potential of the obtained CQDs for FL sensing applications in aqueous samples.[2, 17]

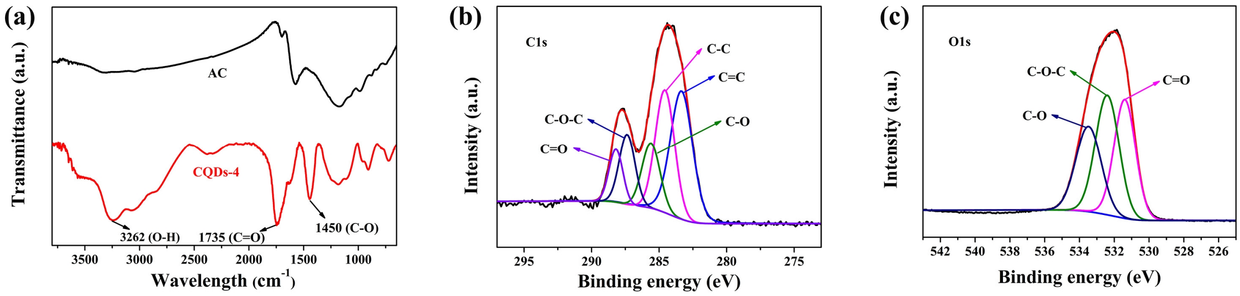

Fig. 2 (a) FTIR spectra of AC and CQDs-4, and XPS curve of CQD-4, (b) C1s, (c) O1s.

To investigate the optical properties of the CQDs samples, UV-vis absorption spectra, FL excitation spectra, and emission spectra were performed. As shown in Fig. 3a, CQDs-4 showed a broad UV absorption at 300 nm and gradually extended to a visible area, which was ascribed to the n-π* transition of C=O. In addition, it had bright yellowish-green FL under the irradiation of 365 nm UV light. The maximum emission wavelength was located at 490 nm by 350 nm excitation wavelength for CQDs-4 with large Stokes shifts of 140 nm. Moreover, the benefit of large Stokes shifts of samples could distinguish the samples from the background signal of instrument in analyzing applications. The FL stability was an important indicator for the obtained CQDs in practical application. To demonstrate the stability of the CQDs-4, FL intensities toward time- and pH-influence were measured. The FL intensities remained almost constant within 60 days, suggesting that the CQDs-4 is highly stable even under long term (Fig. 3b). The CQDs-4 displayed stable FL intensities in the range of pH 3 to 10 (Fig. 3c), which can be understood in terms of the change in surface charge owing to protonation-deprotonation.[18] The CQDs were intrinsically water-soluble because of the oxygen-rich functional groups over the surface. So, the water-solubility and the FL stability of CQDs-4 provided a convenient condition for the actual production application.

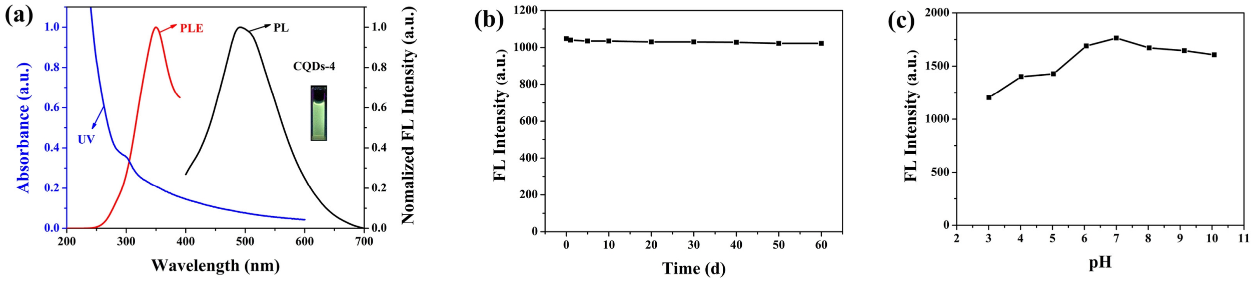

Fig. 3 (a) UV-vis absorption spectra, FL excitation and emission spectra of CQDs-4 (inset: optical images of CQDs-4 illuminated under 365 nm UV light.). (b) FL responses of CQDs-4 with different time at room temperature. (c) FL responses of CQDs-4 at different pH values.

Establishment of CQDs fluorescent probe for Cr^{3+}

We explored the feasibility of using the obtained CQDs for Cr^{3+} detection. Taking the selectivity requirement into consideration, the CQDs-4 was applied to detect different ions in aqueous solution for estimating fluorescent probe. A number of metal ions (Ag^{+}, Al^{3+}, Ba^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Na^{+}, K^{+}, and Cr^{3+}) were tested at a concentration equal to 40 μM excited by 350 nm wavelength. As shown in Fig. 4a, the FL intensities of CQDs-4 were significantly decreased in the presence of Cr^{3+}, and only little changes of the FL intensities were observed in the presence of the other ions that out except Cr^{3+}. The variations of FL intensities may be related to the complexation of Cr^{3+} with the oxygen-containing functional groups on the surface of CQDs-4.[11]

In order to analyze the size effect of the five CQDs samples on the detection of Cr^{3+}, we added the same concentration of Cr^{3+} (40 μM) to the obtained CQDs for observing the FL quenching phenomena. The response rate of the FL intensities was calculated with equation (1) to quantitatively investigate the FL quenching effect of five CQDs samples.

$$\Phi = \frac{F}{F_0} \qquad （1）$$

where Φ, F_0 and F represent the response rate of the FL intensities, the FL intensities of the obtained CQDs in the absence and presence of Cr^{3+}. As shown in Fig. 4b, the FL quenching effect was highly dependent on the sizes of the obtained CQDs, where CQDs-4 with 3.6 nm had the optimal FL detection performance. In addition, the color of the five CQDs samples under the irradiation of 365 nm UV light also changed (Fig. 4c) according to the response rate of the FL intensities.

As the literatures reported, the FL quenching of CQDs was explained on the basis of the complexation of Cr^{3+} with the carboxylic and hydroxyl groups present over the surface of CQDs.[2, 11, 17] In this work, the CQDs had oxygen-rich functional groups as UV-vis and FTIR tested, however, they performed different FL quenching effect. The reasons were that CQDs as fluorescent probe for detecting Cr^{3+} relates to size of CQDs, considering the distribution and spatial repulsion or electrostatic repulsion of surface groups over the CQDs. The FL quenching phenomena of CQDs with different sizes indicated that the size of CQDs played a crucial role in the FL quenching process of CQDs detecting Cr^{3+}, based on the complexation of Cr^{3+} with the functional groups on the surface of

CQDs. Therefore, we proposed that CQDs as efficient fluorescent probe to detect Cr^{3+} depend on the synergistic effect of the surface functional groups and size of CQDs.

To evaluate the sensitive applicability of the CQDs-4 for the trace detection of Cr^{3+}, we investigated the linear response range and the limit of detection (LOD). The FL intensities of the CQDs-4 were obviously declined with the addition of Cr^{3+} (Fig. 4d). Upon the addition of Cr^{3+}, the color of the CQDs-4 solution became colorless from yellowish-brown. Moreover, the FL intensities of CQDs-4 decreased and the emission peak shifted from 490 nm to 480 nm. The slight blue shift of emission peak probably caused by the disappearance of the size-dependent trap states of CQDs-4, as a result of the stronger affinity between Cr^{3+} and CQDs-4.[2] There was a good linear between ΔF and Cr^{3+} concentration in the range of 0.2 to 55 μM, with the linear regression equation, $\Delta F = 1.44 \times 10^7 [Cr^{3+}] + 10$ ($R^2 = 0.9990$), where $[Cr^{3+}]$ was the concentration of Cr^{3+}, as shown in Fig. 4e. The LOD of Cr^{3+} was 0.2 μM calculated based on $3\sigma/k$, where σ was the standard deviation of the blank signal, and k was the slope of the linear plot. The LOD was much lower than the maximum level (1 μM) of Cr^{3+} permitted in drinking water by the WHO.

Considering the complex components of the real sample, we also tested the selectivity of the CQDs-4 for Cr^{3+} over the common ions. Experimental results (Fig. 4f) suggested that the investigated metal ions made no effect on the fluorescent probe performance. The results suggested that the CQDs-4 displayed high selectivity for the detection of Cr^{3+}. The practicability of the CQDs-4 for Cr^{3+} was also assessed by applying it to the real analysis for Cr^{3+} in tap water samples. Four Cr^{3+} concentration points of 15 μM, 25 μM, 40 μM, and 50 μM were added to the tap water samples for testing, the measurement results were shown in Table 1. The recovery of spiked Cr^{3+} over 97% and the relative standard deviation (RSD) is lower than 1.5%. The results indicated that the good practicability of our fluorescent probe for Cr^{3+} determination in tap water samples.

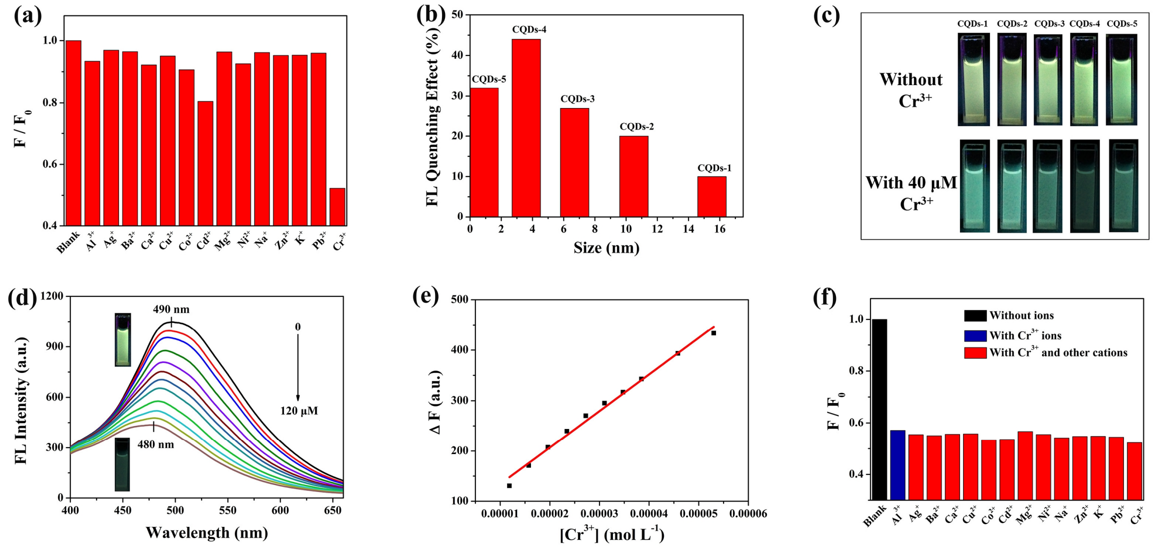

Fig. 4 (a) FL responses of CQDs-4 with 40 μM of different metal ions. (b) The relationship of FL quenching effect and sizes of the five CQDs samples for detecting Cr^{3+}. (c) Optical images of the five CQDs samples illuminated under 365 nm UV light in the absence and presence of Cr^{3+}. (d) FL emission spectra of the CQDs-4 with various concentrations of Cr^{3+}. (e) The linear plot of the FL intensity changes versus Cr^{3+} concentrations ranging from 0.2 to 55 μM. The excitation wavelength was 350 nm. (f) Selectivity of the CQDs-4 fluorescent probe for Cr^{3+} over other metal ions. F_0 and F were the FL intensities of the CQDs-4 in the absence and presence of different ions, respectively.

Table 1 Determination results of Cr^{3+} in tap water samples by using GQDs-4.

Sample	Add of Cr^{3+} (μM)	Found of Cr^{3+} (μM)	RSD (n=3, %)	Recovery (%)
1	15	14.9	0.2	99.3
2	25	24.5	0.3	98.0
3	40	39.3	0.3	98.2
4	50	48.5	0.2	97.0

Conclusions

The yellowish-green fluorescent CQDs with different sizes were prepared using AC as the carbon source with H_2O_2 chemical oxidative method, in which the tuning of size was simply carried out through changing concentration of H_2O_2 aqueous solution. Experimental results showed that the concentration of H_2O_2 aqueous solution played crucial role on the sizes of CQDs. The XPS and FTIR results showed that many oxygen-rich functional groups were on the surface of CQDs, which were beneficial to enhance the hydrophilicity of the CQDs in aqueous solution. The CQDs-4 had been used as highly selective and sensitive fluorescent probe for detecting Cr^{3+} with a lower detection limit of 0.2 μM from 0.2 to 55 μM. The sensitivity of CQDs as fluorescent probe to detect Cr^{3+} depended on the synergistic effect of the surface functional groups and size of CQDs. The obtained CQDs showed good practicability in detecting tap water samples.

Acknowledgements

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