Preparation of carbon dots with orange emission for Cr(III) detection and cellular imaging

1 | INTRODUCTION

Chromium and its compounds, which were widely applied in metallurgy, electroplating, coating and battery industries, have been discharged into the water environment with an increasing amount in recent years [1]. Cr(III) is one of the main ionic forms of chromium element in the environment. For the human health, Cr³⁺ has been identified to be essential to maintain the normal carbohydrate metabolism [2]. However, under the excessive condition, it will hurt the chromosome structure, respiratory system and even lead to cancer [3]. So, it would be of great significance to develop efficient methods to detect Cr(III). At present, the main methods for the determination of Cr(III) are atomic absorption spectrometry, atomic emission spectrometry and X-ray fluorescence spectrometry, etc. [4, 5]. However, there are some problems for these methods, such as high cost, time-consuming and complex operation, which limit their application and development. The detection of metal ions based on fluorescence analysis has the advantages of simple operation, high sensitivity and good selectivity [6]. Therefore, the establishment of new fluorescence methods for Cr(III) detection has as great value both on theory and practice [7].

Fluorescent carbon dots (CDs), a fascinating star of “zero-dimensional” nanomaterials, have received huge attentions owing to their physicochemical properties, such as good water solubility, low biological toxicity, flexible modification [8]. Because of their particular advantages, versatile carbon sources and synthetic routes have been developed to produce CDs for vast applications, such as bioimaging, drug loading and photoelectric materials, etc. [9, 10]. However, most of the reported CDs could emit fluorescence in the blue or green region under excitation with a few exceptions [11]. As we know, the fluorescent nanomaterials with long wavelength absorb/emitted fluorescence might be more popular in the bio-related applications, because they could effectively avoid the influence of autofluorescence and possess excellent optical penetrability in biological structure [12]. As a result, it is of great significance to produce CDs with long wavelength emission for use, especially, biological applications.

In this research, a new type of orange emission carbon dots (OCDs) was prepared by hydrothermal method using 1,2,4-triaminobenzene and p-aminobenzenesulphonic acid as precursors, which are cheap and common chemical regents. Furthermore, the OCDs were found to exhibit excellent PL sensitivity and selectivity for Cr(III) detection, while the potential applications in bio-sensing of Cr(III) at cellular level were explored.

2 | MATERIALS AND EXPERIMENTAL

1,2,4-triaminobenzene and p-aminobenzene sulphonic acid were purchased from Shanghai Aladdin Reagent Co., Ltd., anhydrous ethanol and various metal salts were purchased from Tianjin Guangfu Chemical Co., Ltd., and the experimental water was secondary distilled water. In addition, Qingdao Guda CY-10AC freeze-dryer was used for freeze-drying of carbon samples.

Transmission electron microscopy (TEM) photographs were performed using a Tecnai G2 F20 TEM with a voltage of 200 kV. The infrared absorption spectra of RCDs were measured by Nicolet 360 FT-IR infrared spectrometer in the range of 480–4000 cm⁻¹. X-ray photoelectron spectroscopy (XPS, Shimadzu) was adopted to analyse the surface chemical structure of the samples. UV–vis spectrophotometer was used to determine the absorption spectrum of the samples by a PERSEE T10CS UV–vis spectrometer. Fluorescence spectra were determined by an FLS-980 fluorescence spectrometer.

0.5 g 1,2,4-triaminobenzene and 0.589 g p-aminobenzene sulphonic acid were dissolved in 60 mL water and stirred to form a solution. The solution was transferred to a stainless steel reactor (100 mL), while the reactor was cooled to room temperature after 8 h of reaction at 180 °C. Subsequently, the reaction product system was centrifuged at 13,000 rpm for 8 min, and the gray-brown precipitate was discarded. Then, the red-brown supernatant was filtered with 0.22 um ultrafiltration membrane to obtain the solution of OCDs. After freeze-vacuum drying, the solid powder of brown OCDs could be obtained for further applications.

Typically, 50 μL Cr(III) solutions with different concentrations were separately added into 3.0 mL Tris-HCl buffer (pH = 7.0) containing 200 μg/mL of OCDs. After incubating at room temperature for 3 min, the fluorescent spectra were...
As shown in Figure 1, the OCDs are highly monodisperse in water and almost spherical in shape with an average size of approximately 4.6 nm. As additional shown from the inset of Figure 1, the high-resolution TEM (HRTEM) image presented that most of the OCDs are amorphous particles without any distinct lattice [15].

In order to investigate the surface chemical structure of the obtained OCDs, the samples were further characterized by FTIR and XPS. As shown in Figure 2, the absorption regions from 3550 and 3200 cm\(^{-1}\) are assigned to the vibrations of N–H and O–H bonds on the surface of OCDs [16]. Broad absorption regions at 2923 and 2850 cm\(^{-1}\) are attributed to the stretching and bending vibration bands of C–H [17]. The obvious and sharp peak appeared at 1635 cm\(^{-1}\) is assigned to the C=O group. The absorption peaks at 1550 cm\(^{-1}\) are resulted from the bending vibration of N–H.

As presented in Figure 3(a), the full XPS spectrum of OCDs clearly exhibits four obvious peaks at 169.0, 284.6, 401.2 and 532.1 eV, which suggested that the OCDs were mainly consisted by S, C, N and O elements, while the contents were calculated to be 1.00%, 75.40%, 15.14% and 8.56%, respectively. The high-resolution spectrum (Figure 3(b)) indicates the C 1s band could be deconvoluted into five regions at 284.6, 285.7, 286.1 and 278.8 eV, which corresponded to C–C/C=O, C–N, C–O, and C=N/C=O, respectively [18]. Based on the FTIR and XPS characterization results, it could be confirmed that the OCDs were constituted of large p-conjugated domains in cores and functional group regions on their surfaces.

The prepared OCDs were also investigated by UV–vis absorption analysis. As presented in Figure 4(a), the UV–vis absorption spectrum shows two noticeable peaks at 251 and 508 nm. For the first peak with higher energy, it is considered to be originated from the \(\pi-\pi^*\) transition of the C=C bonds, which normally emit competitively short-wavelength fluorescence [19]. For the 508 nm absorption peak, which might be originated from the surface states with C=N/C=O and C–O structures [20]. Such unique absorption characters might cause experiments were conducted five times, while the values were recorded as the mean ± standard deviation.

In a typical process, 1.0 mL of HeLa cells cultured in Dulbecco’s modified Eagle’s medium (DMEM, 2 \(\times\) 10\(^5\) cells per well) were seeded in 6-well plates and incubated according to previous reports [14]. Then, the DMEM was removed, and a mixture of RCDs (200 \(\mu\)g/mL) in DMEM was added in each well. After 2 h incubation, the cells were washed twice with PBS solution (pH = 7.4) to remove the free OCDs and then fixed with 4% (v/v) paraformaldehyde for 30 min at 4°C. Cellular fluorescence experiments were performed using an Olympus-IX73 inverted fluorescence microscope.

For assessing Cr(III) uptake, the cells were incubated with OCDs at a concentration of 250 \(\mu\)g/mL for 24 h as described, followed by treating with CrCl\(_3\) (250 \(\mu\)g/mL) for 10 min at normal temperature.

### 3 RESULTS AND CONCLUSION

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Figure 3. XPS of the obtained OCDs. (a) XPS survey scan spectrum. (b) High-resolution XPS spectra of C1s of the OCDs.

Figure 4. Optical analysis results of the OCDs in aqueous solution. (a) UV–vis absorption spectrum. (b) PL emission spectra under different EX wavelength (450–550 nm) of OCD samples.

long-wavelength emission under excitation for the obtained samples. In addition, from the digital images inserted in Figure 4(a), it could be seen that the prepared OCD aqueous solution is brown under natural condition, which is corresponding to the features of UV–vis absorption spectrum. Upon laser irradiation at 520 nm, the OCD aqueous solution generated a beam of orange-coloured light.

Subsequently, the fluorescent characteristics of the OCDs were analysed. As shown in Figure 4(b), the obtained samples exhibit excitation-independent fluorescence behaviour, with a maximum emission peak at approximately 589 nm. At the optimal 500 nm excitation, the absolute fluorescence quantum yield of the OCDs was 14.9% by integration sphere method. In order to evaluate the practical application value, the durability of the OCDs was further explored. The OCDs showed excellent photostability, and the PL intensity of OCDs kept almost constant under continuous irradiation (365 nm) for 80 min with an Xe lamp (Figure 5).

Further, the sensing performance of the obtained OCDs to Cr(III) was discussed. Interestingly, the obtained OCDs are found to show an interesting “on-off” fluorescence response with the addition of Cr(III). As shown in Figure 6(a), the quenching efficiency \((F_0 - F)/F_0\) displayed an excellent linear relationship with the concentration of Cr(III) in the range of 1.0–96.0 μmol/L μM with a good correlation coefficient of 0.9941. The fitted linear regression equation is calculated as \((F_0 - F)/F_0 = 0.0061x + 0.1229\), where \(F_0\) and \(F\) were the FL intensities of the OCDs in the absence and presence of Cr(III), respectively. The detection limit for Cr(III) was found to be 0.38 μmol/L on the basis of the three times signal-to-noise criteria [21].

For evaluating the selectivity of the OCDs toward Cr(III), the effects of some potential interfering substances, including various metal ions, such as Na(I), Ca(II), Mg(II), Al(III), Fe(II), Fe(III), Zn(II), Cr(III), Co(II), Mn(II), Hg(II), Ag(I), were investigated. As shown in Figure 6(b), most common interferents, except Fe(III) and Co(II), have no significant influence on the fluorescence intensity of OCDs.

The possible mechanism of the fluorescence response of OCDs to Cr(III) was further explored. As shown in Figure 7, the average FL lifetime of the OCDs was calculated to be 1.86 ns, after the reaction with Cr(III), the average FL lifetime...
The results of the cation detection experiment. (a) The relationship of \((F - F_0/F_0)\) against increasing Cr(III) concentrations (0.5–140 μmol/L). (b) Comparison of fluorescence intensities of OCDs after the addition of different metal ions (concentration: 0.1 mmol/L).

**FIGURE 7** Fluorescence decay trace of OCDs before and after (inset) the addition of Cr(III); The measurement was made at \(\lambda_{em} = 560\) nm

was changed to be 1.74 ns. It could be seen that the average FL lifetime remains almost constant, which indicated that the fluorescence quenching was probably attributed to static fluorescence quenching process. What is more, this phenomenon might be caused from the chelation between surface polar groups of OCDs and Cr(III), which promoted the charge transfer from the excited state of OCDs to Cr(III) [22]. To prove this possible interaction mechanism, UV/Vis absorption analysis were further conducted. As shown in Figure 8, the absorption peaks of the OCDs redshifted (from 500 to 583 nm) after adding Cr(III), which proves that Cr(III) indeed complexed with the OCDs [23].

Comparing the performance of the as-prepared OCDs with the recently reported CDs in sensing ions (Table 1), it could be seen that the sensitivity of the sensor was comparable in the fluorescent manner.

In order to evaluate the biological applications of OCDs for the determination of Cr(III) in living cells, the cytotoxicity against HeLa cells was investigated using MTT colorimetric assays. As shown in Figure 9, 92.5% cell viability was observed after incubating HeLa cells with OCDs (from 0 to 300 μg/mL) even for 36 h. So these OCDs exhibited low cytotoxicity and good biocompatibility to living cells [29], which indicated that the OCDs were novel carbon materials with excellent health friendliness.

The cell imaging applicability of the OCDs was investigated by confocal microscopy experiments. After incubating HeLa...
cells treated with OCDs in PBS for 8 h at 37 °C significant red fluorescence could be observed (Figure 10(b)), which was mainly presented in the nucleus region. Followed by introducing 2 mM Cr(III) into the ORCDs-pretreated HeLa cells, as expected, the intracellular fluorescence was distinctly induced (Figure 10(d)), which suggested that the OCDs could be a promising labelling agents for Cr(III) detecting in cell level [30].

4 | CONCLUSION

In summary, OCDs were successfully prepared by hydrothermal treatment of 1,2,4-triaminobenzene and p-aminobenzenesulphonic acid. These OCDs emitted excellent and excitation-independent red luminescence in aqueous solution. Moreover, the OCDs could be served as an PL probe for effective and rapid sensing detection of Cr(III) ion, with a low detection limit of 0.38 μM. In addition, the OCDs exhibited low cytotoxicity, which allowed them to be applied for imaging intracellular Cr(III). These novel OCDs provide a promising nanomaterial platform for efficient biological applications.

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