Green synthesis of N-doped carbon quantum dots for the detection of nitrite ion in water sample

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Abstract. In this work, a green hydrothermal method was used to synthesize nitrogen-doped carbon quantum dots (N-CQDs) from Ecuadorian shrimp shell. X-ray diffraction (XRD) pattern showed that N-CQDs had an amorphous carbon peak. Both Fourier transform infrared (FT-IR) spectra and X-ray photoelectron (XPS) spectra indicated the presence of C, O and N in N-CQDs. The elemental analysis revealed that the weight percentage of C, H and N in N-CQDs are 45.7\%, 6.9\% and 13.2\%, respectively. Transmission electron microscopy (TEM) image demonstrated N-CQDs had an average size of 3 nm and spherical shape. While quinine sulfate with 54\% fluorescent quantum yield was used as a reference, the quantum output of N-CQDs was calculated to be 23.7\%. Ultraviolet-visible absorption spectra of N-CQDs showed that the maximum absorption wavelength was 275 nm. Photoluminescence (PL) spectra of N-CQDs displayed highest PL intensity at excitation and emission wavelengths of 323 and 407 nm, respectively. The results showed that the synthesized samples were N-CQDs. N-CQDs can be used as a fluorescent probe for nitrite ions in water. However, nitrite ion was not detected in Hu County River water and Xi’an tap water. This may be due to the absence of nitrite ion in the selected water samples.

1. Introduction
Carbon quantum dots (CQDs) are novel nanomaterials with small particle sizes and unique physical-chemical properties [1]. Since their discovery, they have become a research hotspot in the field of materials. Among the characteristics of CQDs, its unique fluorescence performance has attracted the attention of researchers. CQDs have been widely applied in the fields of biological imaging and fluorescence detection. However, the fluorescence yield of common CQDs is relatively low, restricting its further application [2,3]. In order to increase the fluorescence yield of CQDs, CQDs are typically surface modified or doped. Previous studies have shown that surface modification of CQDs using polyethylene glycol polymers can increase the fluorescence yield by 15\% [4]. Although surface modification can improve the fluorescence yield of CQDs to some extent, the doping of CQDs can obtain better performance. Li et al [5] found that N-CQDs not only have high fluorescence yield, but also show excellent performance. Therefore, N-CQDs have become a new type of carbon quantum dot nanomaterials with research significance.

Nitrite ion is a common-existing inorganic substance in aqueous system. It is widely concerned due to its carcinogenic nitrosamines attribute. Therefore, developing rapid, simple and accurate detection method of nitrite ions is necessary. According to World Health Organization standards, the concentration of NO\textsubscript{2} in drinking water should be less than 65 \mu M [6]. For the detection of nitrite ions, high-efficiency chromatography [7], colorimetry [8,9], electrochemistry [10] and other detection
methods are generally used. Among them, the fluorescence detection method has become a hot research technology owing to the merits of fast detection speed, high sensitivity and easy operation.

In this study, fluorescence N-CQDs were prepared by green hydro-thermal synthesis reaction way using Ecuadorian shrimp as raw materials, the preparing process does not need costly chemicals, high temperature, and strong acid [11-13]. Furthermore, the feasibility of using N-CQDs to detect NO\textsubscript{2}\textsuperscript{-} in aqueous media was verified and the detection application of N-CQDs in actual water samples was studied.

2. Experimental

2.1. Synthesis of N-CQDs

In this experiment, the Ecuadorian Shrimp shell was used as starting materials. First, fresh Ecuadorian Shrimp shell was washed with water, dried in a drying oven at 50\degree C for 1 day and then grounded into powder. Secondly, 7.5 g Ecuador shrimp shell powder and 30 mL purified water were placed into 50 mL Teflon lined stainless steel autoclave for hydrothermal reaction. The Teflon-lined stainless autoclave was sealed and heated in a convection oven at 180\degree C for 12 h. The obtained suspension was purified by 0.22 μm filters. The filtrate was then centrifuged at 4500 rpm and 11000 rpm for 15 min, respectively. Finally, the supernatant was dialyzed. Dry the dialysis solution and the desired N-CQDs were obtained.

2.2. Characterization

X-ray diffraction pattern of the prepared N-CQDs was recorded by a Shimadzu XRD-7000s diffractometer in the 2θ range 10-80° with a scanning speed 0.02°/sec. The TEM image of N-CQDs was investigated by a JEM-3010 TEM. FT-IR spectrum of N-CQDs was recorded by a FT-IR 8000 photometer from SHIMADZU. The Ultraviolet-visible absorption curve of the N-CQDs solution was determined by a UV-2102 PC Spectrophotometer. Fluorescence spectra of N-CQDs solution was measured by an RF-6000 Spectrofluorophotometer. XPS data of N-CQDs was recorded by a Kratos AXIS-ULTRADLD X-ray photoelectron spectrometer. The obtained XPS report was analyzed through the XPS Peak 4 Software. Vario Micro Element Analyzer was used to simultaneous determine C, Hand N in N-CQDs.

2.3. Detection of nitrite ion

In order to ensure the accuracy and reproducibility of the experiment, the detection of NO\textsubscript{2}\textsuperscript{-} was performed in 0.2 M phosphate buffer solution (PBS, pH=7.0) [14]. The possibility of detection was verified by measuring the fluorescence intensity at the excitation wavelength (323 nm) of 0.2 M PBS, N-CQDs mixed with 0.2 M PBS, 1.0 mM NO\textsubscript{2} mixed with 0.2 M PBS, N-CQDs mixed with 1.0 mM NO\textsubscript{2} and 0.2 M PBS. And the time dependence of NO\textsubscript{2} solution on fluorescence quenching of N-CQDs was also researched by detecting the fluorescence intensity of N-CQDs from adding NO\textsubscript{2} to quenching balance in 0.2 M PBS. The effects of different concentrations of NO\textsubscript{2} solution on fluorescence performance of N-CQDs was detected by measuring its fluorescence intensity in PBS solutions in various concentrations of NO\textsubscript{2}.

3. Results and discussion

3.1. Characterization

The chemical and physical properties of the prepared N-CQDs were characterized by a series of experiments. Powder X-ray diffraction analysis was performed to determine the crystal structure of N-CQDs. As shown in figure 1(a), an amorphous peak around 20.7° was observed, corresponding to graphitic [002] plane [15]. Fourier transform infrared spectra further revealed the chemical structure of N-CQDs. The broad band at 3292 cm\textsuperscript{-1} was ascribed to N-H vibration. The bands at 3070 cm\textsuperscript{-1} and 2964 cm\textsuperscript{-1} were corresponding to C-H stretching vibration of -CH\textsubscript{2} and -CH\textsubscript{3}, respectively. The sharp
peak at 1651 cm\(^{-1}\) was attributed to C=O vibration. The peak at 1543 cm\(^{-1}\) was corresponding to -NO\(_2\) antisymmetric vibration. The peaks at 1448 cm\(^{-1}\) and 1404 cm\(^{-1}\) were corresponding to rocking of -CH\(_2\) and -CH\(_3\). The peaks at 1242 cm\(^{-1}\) and 1039 cm\(^{-1}\) were derived from C-O stretching vibration.

![Figure 1](image1.png)

**Figure 1.** (a) XRD pattern and (b) FT-IR spectra of N-CQDs.

The morphology of the synthesized N-CQDs was recorded by TEM. As shown in figure 2(a), N-CQDs were sphere-like with uniform distribution. The average size of N-CQDs was calculated to be 3 nm. The high resolution TEM image of N-CQDs was displayed in figure 2(b). The lattice spacing of N-CQDs was measured to be 0.3 nm, which was corresponding to the [002] plane. The obtained results were consistent with the XRD analysis.

The chemical composition of the prepared N-CQDs was determined by X-ray photoelectron spectrometer. As shown in figure 3(a), distinct C, N and O peaks in XPS survey were observed at 283 eV, 397 eV and 529 eV, which was corresponding to C1s, N1s and O 1s, respectively. The two peaks located at 285.7 eV and 282.3 eV in figure 3(b), suggesting C-N and C-C groups existed in the synthesized N-CQDs [16,17]. The high resolution XPS curve of N1s was shown in figure 3(c). The peak located at 397.4 eV was derived from nitrogen of pyridine [17]. The high resolution XPS curve of O1s was shown in figure 3(d). The peak located at 529.3 eV was derived from C=O group in the synthesized sample [18]. Furthermore, elemental analysis was carried out to confirm the existence of N, C and H in the synthesized quantum dots. The weight percentage of N, C and H were calculated to be 13.2%, 45.7% and 6.9%, respectively. Thus, both methods verified the presence of N element in the synthesized quantum dots.

![Figure 2](image2.png)

**Figure 2.** (a) TEM image and (b) high resolution TEM image of N-CQDs.
The photology attribute of N-CQDs were studied by ultraviolet-visible and fluorescence spectra. As shown in figure 4(a), the maximum ultraviolet absorption wavelength of N-CQDs was 275 nm. This blue absorption peak was derived from the aromatic structures of N-CQDs [19]. The color of the N-CQDs solution was light yellow under natural light, whereas intense blue fluorescence was seen under UV lamp irradiation with a wavelength of 365 nm. The recorded pictures of the N-CQDs solution in a cuvette under two different conditions were shown in the inset of figure 4(a). Moreover, detailed fluorescent attribute of the N-CQDs solution under different excitation wavelength in increments of 10 nm was shown in figure 4(b). Under excitation wavelength from 303 nm to 383 nm, the photoluminescence peaks were ranging from 401 nm to 451 nm. The maximum photoluminescence emission peak was measured at 407 nm, when the excitation wavelength was 323 nm. Thus, in the following detection experiment, the excitation wavelength was set as 323 nm. Quantum yield measurement was further carried out to determine the synthesized quantum dots’
photoluminescence attribute. Using quinine sulfate of 54% as a reference, the quantum yield of N-CQDs was calculated to be 23.7%. Fluorescence quantum yield of the synthesized N-CQDs was higher than most previously reported N-CQDs. The obtained results from the above experiments showed that the prepared N-CQDs had excellent fluorescence properties and could be used as a fluorescence probe in the following detection experiment.

3.2. Detection of nitrite ion
The detection of nitrite ion experimental results were shown in figure 5(a). Photoluminescence response was not detected in the PBS with or without NO$_2^-$ . In the PBS containing N-CQDs, the photoluminescence intensity of the solution containing NO$_2^-$ was significantly lower than that of the solution without NO$_2^-$, which proved that N-CQDs had great detection efficiency of NO$_2^-$ with a concentration of 1.0 mM. The time course of the photoluminescence intensity of N-CQDs was further investigated. Figure 5(b), NO$_2^-$ could quickly quench the fluorescent light of N-CQDs, and the photoluminescence intensity basically reached a stable state in 5 min, which means that the N-CQDs and NO$_2^-$ reached a balance state below the quenching agent (1.0 mM, NO$_2^-$) with a given concentration. As shown in figure 5(c), a NO$_2^-$ solution with a concentration gradient of 0-20 mM was prepared, and its effect on the photoluminescence intensity of the N-CQDs solution was measured. Figure 5(d) showed the relative PL response of different NO$_2^-$ concentrated in N-CQDs solutions. The results showed that the PL response of N-CQDs solution decreased linearly with the increase of NO$_2^-$ concentration.

![Figure 5](image_url)

**Figure 5.** (a) Photoluminescence response of four solutions, (b) The time dependence of NO$_2^-$ solution on fluorescence quenching of N-CQDs, (c) Photoluminescence response of different NO$_2^-$ concentrations and (d) Relative PL response of N-CQDs solutions with different NO$_2^-$ concentrations.

3.3. Recovery experiment of nitrite ion
The standard curve of the relative relationship between NO$_2^-$ concentration and fluorescence intensity
was calculated in the range of 0.1-0.8 mM (figure 6). In order to investigate the use of the synthesis N-CQDs detect NO$_2^-$ with fluorescence method in actual waste water, this experiment chose Xi'an tap water and hu county river water as samples. However, NO$_2^-$ was not detected in the selected water samples. This may be because the NO$_2^-$ concentration is under the detection line in the two water specimens, or there is no NO$_2^-$. Running water and river water specimens were served as the substrate for recycling experiments to verify the developed method. The experimental results were shown in table 1. As listed in table 1, the recovery values in tap and surface water varied from 94% to 106% and 94% to 101%, respectively. The recovery values and relative standard deviation met with standards, suggesting the developed method was suitable for detection of NO$_2^-$ in actual water samples.

![Figure 6](image-url)  
Figure 6. The calibration curve of NO$_2^-$ concentration versus fluorescence intensity.

**Table 1.** Recovery experiment of NO$_2^-$ in tap water and surface water.

| Sample     | NO$_2^-$ concentration added (μm) | NO$_2^-$ concentration detected (μm) | Recovery rate (%) | Relative standard deviation (%) |
|------------|----------------------------------|-------------------------------------|-------------------|-------------------------------|
| Tap water  | 30                               | 31.7                                | 106               | 2.6                           |
|            | 100                              | 93.9                                | 94                | 1.7                           |
|            | 500                              | 484                                 | 97                | 2.6                           |
|            | 30                               | 28.1                                | 94                | 2.8                           |
| Surface water | 100                          | 101                                 | 101               | 2.6                           |
|            | 500                              | 496                                 | 99                | 1.8                           |

4. Conclusions

In this study, N-CQDs were prepared by an environmentally friendly hydrothermal reaction using Ecuadorian shrimp shell as raw material. In the nitrite detection and recovery experiment, real water sample was used as a matrix. It was confirmed that the synthesized N-CQDs was sensitive to nitrite ions, and the response had a linear relationship with the concentration of NO$_2^-$. The conclusions demonstrated the feasibility of synthesized N-CQDs using Ecuadorian shrimp shell and its application on the rapid detection of NO$_2^-$ ions in surface water samples.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (21706207).

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