One-step Hydrothermal Synthesis of N-doped Fluorescent Carbon Dots from Fermented Rice with Highly Selective Characteristics for Label-free Detection of Fe$^{3+}$ Ions and as Fluorescent Ink

Yanli CHEN,*† Xueling SHAN,* Ding JIANG,* and Xiaoge LI**

*Jiangsu Key Laboratory of Advanced Catalytic Materials and Technology, School of Petrochemical Engineering, Changzhou University, Changzhou 213164, P. R. China
**Key Laboratory of Mesoscopic Chemistry of MOE, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P. R. China

In our work, N-doped carbon dots (CDs) were synthesized by a facile hydrothermal method with fermented rice as the carbon source. The CDs show bright blue fluorescence, and the maximum emission wavelength was 380 nm with wavelength ranges from 320 to 560 nm. Interestingly, these as-prepared CDs show strong blue photoluminescent properties under the radiation with ultraviolet (UV) light. Moreover, it also exhibits good sensitive fluorescence detection for Fe$^{3+}$ ions; the detection limit is 0.1 μM, which is significant fluorescence quenching based on CDs. Other representative metal ions were further tested to verify their selectivity, which provides a solid underpinning for the practical use in Fe$^{3+}$ ions detection in real samples, e.g. underground water. In addition, the CDs work well as a fluorescent ink and can encrypt and store information.

Keywords N-doped carbon dots (CDs), fermented rice, Fe$^{3+}$ detection, fluorescent probe

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As a fundamental element of life, Fe$^{3+}$ plays an indispensable role in many chemical and biological processes; for instance, hemoglobin formation, oxygen transport, and muscle and brain functions. Because it can not only provide the oxygen-carrying capacity of heme, it also plays an auxiliary role in many enzymatic reactions. However, an unbalanced content of Fe$^{3+}$ can lead to anemia, intelligence decline, Alzheimer’s disease, and other neurodegenerative diseases. Thus, selective and sensitive Fe$^{3+}$ detection may offer a broad prospect for an early diagnosis and treatment. Until now, various examples of Fe$^{3+}$ detection have been reported. However, these methods have some disadvantages, for example, the need for additional particle surface modification procedures, time-consuming sample pretreatment procedures, expensive instruments, or the toxicity and potential health and environmental risks. Therefore, it is urgent to develop new nontoxic optical sensing nanomaterials as well as a simple, sensitive and selective Fe$^{3+}$ detection method.

Due to its wide range of applications in adsorption, separation, electrochemistry, and wastewater treatment, carbon is a popular material that has attracted much attention and has been systematically studied. Carbon-based dots can show excellent optical properties, such as photoluminescence (PL), electrochemical luminescence (ECL), and photo-induced electron transfer. More importantly, compared with semiconductor-based quantum dots containing heavy metals, it also has the advantages of easy preparation, great water solubility, high chemical stability, low-toxicity, excellent biocompatibility, and being environmentally friendly. These outstanding properties together with their small size, near isotropic shape, surface traps, edge states and rich hydrophilic groups on their surface make fluorescent CDs, especially heteroatom doped CDs very promising substitutes of semiconductor-based quantum dots. It is worth mentioning that functional groups and surface defects on CDs have great influence on the luminescent characteristics and applications of CDs. In addition, heteroatom doped CDs can not only improve their fluorescence efficiencies, but also provide active sites in CDs, thus expanding their potential applications. CDs have attracted considerable attention focused on their applications in bioimaging, biosensing, biological labelling, catalysis, optoelectronic devices, photoreduction of metals.

This backdrop has led to its extensive research. As a result, many methods for synthesizing environmentally friendly CDs have been developed. Generally, the strategies for synthesizing CDs are top-down methods. This includes arc discharge, laser ablation, laser irradiation, or electrochemical oxidation, where the CDs are stripped from larger carbon materials, for example, graphite, graphite oxide, carbon nanotubes, and activated carbon. There are also reports on bottom-up methods for synthesizing CDs from some molecular precursors, for example, citric acid, carbohydrates, carbon soot, and resin, through combustion, thermal decomposition, strong acid oxidation, dehydration, or microwave treatment, and so on. Among these, a hydrothermal method provides a low-cost, convenient, nontoxic, and an eco-friendly method to synthesize CDs from biomass.

To the best of our knowledge, nature offers an almost infinite resource, which is easily available for material scientists to replace conventional chemicals for biological inspiration to prepare materials with new structures and properties. Some successful demonstrations have been given to synthesize...
nanomaterials from natural biomass, such as grass,55 pomelo peel,56 soy milk,57 papaya,58 mangosteen pulp,4 rose-heart radish,59 and cedrus plant.60 It is worth mentioning that these carbon sources are low-cost, and sustainable, which encourage us to search for new carbon sources. It is well known that rice is particularly high in carbon, nitrogen, and oxygen elements due to the presence of carbohydrate, lipids and proteins. This natural source is expected to be an alternative precursor for the synthesis of excellent nanomaterials.

In this article, we develop an easy and cheap method to synthesize N-doped and water-soluble CDs through a hydrothermal treatment of fermented rice. Carbonization will take place in the process of the hydrothermal treatment, which results in the formation of N-doped CDs. Furthermore, it was discovered that the prepared N-doped CDs exhibit excellent water-dispersibility and strong photoluminescence (PL). Their PL stability in NaCl aqueous solution with different concentrations illustrates the potential application, as shown here to be an excellent PL sensing system for detecting Fe3+. On this basis, it has been used in the detection of Fe3+ in underground water samples successfully. What is worth mentioning is that this method is cheap, facile and applicable for large-scale synthesis. We believe that these environmentally friendly CDs will be mass-produced cheaply for commercial use.

**Experimental**

**Preparation of fermented rice**

The processing of fermented rice was so simple that it was easy to make even at home. First, 2 – 3 cups of the steam rice were scooped into a bowl. We made sure it was warm, not hot. And second, we added yeast to a bowl, but before that the yeast was ground into a powder. Then, a food wrapper was put over the bowl of yeast and rice. Finally, we put it in a warm corner separated from one another. The distribution of the diameters was separated from one another. The distribution of the diameters within the range of 5.0 – 7.9 nm, and the average particle diameter is 6.4 nm. The XRD analysis of CDs, as shown in Fig. 1b, exhibits a wide band (002) at around 21.72° corresponding to the typical 0.409 nm interlayer spacing.61

**Preparation of CDs**

CDs were prepared by using the hydrothermal treatment of fermented rice. We added 3 g of fermented rice to 30 mL of deionized H2O. The mixture was moved to a 50 mL Teflon-lined steel autoclave and heated at 180°C for 24 h. After cooling to ambient temperature, the suspension was collected and centrifuged at 3000 rpm for 3 min. The insoluble substances were then removed and a pale-yellow supernatant containing fluorescent CDs was obtained.

**Fluorescence assay of Fe3+**

We added different concentrations of Fe3+ solutions (0.7 mL) to the as-prepared CDs (1.4 mL). After 5 h, the PL emission spectra were recorded at room temperature. To evaluate the selectivity of Fe3+ detection, changes in the PL intensity were also tested in the presence of other typical metal ions under the same conditions.

**Fluorescent ink**

We selected filter paper with no background under UV fluorescence as the typing paper. Pale-yellow CDs were then put into a pen for writing different patterns on the filter paper.

**Characterization**

A Varian Cary Eclipse fluorescence spectrophotometer was used to obtain PL spectra. The morphologies of CDs was studied by JEOL JEM-2100 transmission electron microscopy (TEM). X-ray diffraction (XRD) analysis was carried out by a Bruker D8Advance diffractometer (Germany) in the range from 10 to 90° (2θ) with Cu-Kα radiation (λ = 1.54056 Å). X-ray photoelectron spectroscopy (XPS) data were performed on an AXIS ULTRA spectrometer (Kratos Analytical Ltd., UK). Fourier Transform Infrared (FTIR) spectrum was taken on a FTS NEXUS spectrometer (Thermo Nicolet Corporation, USA). Raman spectra were obtained on a confocal Raman microscope (Renishaw inVia Microscope, UK) using 532 nm wavelength incident laser light. A TU-1810 UV-vis spectrophotometer (Pgeneral, China) was used to record UV-visible absorption spectra.

**Results and Discussion**

**Structural characterizations of CDs**

A yellow dispersion solution was obtained by a hydrothermal treatment of fermented rice at 180°C. Figure 1a shows the TEM image of CDs, showing that they are composed of CDs well separated from one another. The distribution of the diameters within the range of 5.0 – 7.9 nm, and the average particle diameter is 6.4 nm. The XRD analysis of CDs, as shown in Fig. 1b, exhibits a wide band (002) at around 21.72°, corresponding to the typical 0.409 nm interlayer spacing.61

Figure 2a shows the XPS survey spectra of CDs within the range of 0 to 1200 eV. The three peaks located at 284.0, 398.0, and 531.0 eV, respectively, belongs to C1s, N1s, and O1s.55 This XPS full-scan spectrum indicates that the nanoparticles are composed mainly of carbon and oxygen, as well as a small amount of nitrogen. The N1s spectrum suggests that the nitrogen had entered the carbon skeleton successfully. Figure 2b shows that the C1s spectrum has four components at 284.5, 286.0, 286.6, and 288.1 eV, which are respectively associated with C-C, C-N, C-O, and C=O.55,62 The N1s spectrum is shown in Fig. 2c. The three component peaks with binding energies of

![Typical TEM image of CDs.](image1.png)

![X-ray diffraction pattern of CDs.](image2.png)

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**Fig. 1** (a) Typical TEM image of CDs. (b) X-ray diffraction pattern of CDs.
about 399.5, 400.5, and 401.5 eV are separately related with C–N–C, N–(C)₃, and N–H.⁵⁵ The nitrogen atom can replace the carbon atom at the edge or the plane of the defect position. Doping of nitrogen atoms can introduce electrons or holes to CDs, which can greatly enhance the electrical structure and chemical activity, and thus improves the fluorescence quantum yield (QY) and PL intensity of CDs.⁶³ The O 1s spectrum (Fig. 2d) presents two components centered at 531.7 and 533.0 eV, corresponding to C=O and C–OH/C–O–C groups, respectively.⁵⁵ The above results indicate that the product may consist of graphitic sp² carbon atom nanocrystalline nucleus functionalized by peripheral carboxyl/carbonyl groups and sp³ carbon defects. All of these observations confirm that there are a large number of hydrophilic groups on the surface of CDs, so they have excellent water-solubility. In Fig. 2e, the peak of FTIR spectrum at 3413 cm⁻¹ corresponds to the –OH stretching vibration. The characteristic peak at ∼1701 cm⁻¹ illustrates the presence of the C=O group for CDs. These data reveal that the obtained CDs contains rich carboxyl groups. The peak value of conjugated C=C stretching vibration was observed at 1635 cm⁻¹, indicating that unsaturated carbon bonds are formed during carbonization.⁶⁴ Moreover, the symmetric and asymmetric stretching vibration of C–O–C (1202 and 1300 cm⁻¹) also show that partially oxidation occurred on the CDs surface during the hydrothermal treatment process.⁴⁴,⁶⁵ The bands at 2971 and 2928 cm⁻¹ correspond to C–H stretching vibration.⁶⁷ Additionally, the characteristic peaks at 1567 and 1425 cm⁻¹ are assigned to N–H bending vibration and C–N stretching vibration, respectively.⁶² This fits nicely with the XPS result. All of these observations prove that the synthesized nanoparticles are rich in hydrophilic functional groups (hydroxy and carboxyl groups), which may be derived from carbohydrates in rice, and are consistent with good aqueous dispersibility. It is a general method to introduce hydrophilic groups into CDs to enhance the solubility and to segregate graphite nanodomains to induce fluorescent properties. In addition, the presence of oxygen-rich groups makes CDs soluble in water media, providing additional advantages for further functionalization to meet different demands.⁶⁵

As shown in Fig. 2f, the Raman spectrum further confirmed the existence of unsaturated carbon bonds. The wide band at 1377 cm⁻¹ is related with the D band, belonging to the sp³ defects.⁶⁶ The first-order G band assigned to the C sp² atoms in-plane bond-stretching is at 1582 cm⁻¹ and the full width at half maximum (FWHM) is ∼111 cm⁻¹. It is proved that the as-prepared CDs contains aromatic and olefinic groups.⁴⁴ The position and width of the D and G bands can be used as indicators of the graphitization degree.⁶⁷ The I_D/I_G ratio (integral
which is the second harmonic of the D line. All of the sites on various size nanoparticles in the sample. Note that can impact the band gap, or the influence of diverse emission peak may be due to the surface state of CDs, which by changing the excitation wavelength. The red shift of the property, showing that the emission wavelength can be adjusted wavelength gradually changes with the change of the excitation wavelength and the inset can clearly elucidates the dependent wavelength.

**Optical properties**

Figure 3a shows the UV-vis absorption spectrum, displaying two characteristic absorption peaks at 227 and 284 nm originating from the $\pi-\pi^*$ transition of graphitic sp$^2$ domains and n-$\pi^*$ absorption band, respectively. What excites us is that the prepared CDs can emit strong blue fluorescence under ultraviolet radiation without any further treatment. This illustration shows pictures of CDs illuminated in daylight (left) and under ultraviolet lamp (right, 365 nm). PL studies at different excitation wavelengths (320 – 560 nm) were carried out, as shown in Fig. 3b. Interestingly, the PL emission wavelength gradually changes with the change of the excitation wavelength and the inset can clearly elucidates the dependent property, showing that the emission wavelength can be adjusted by changing the excitation wavelength. The red shift of the emission peak may be due to the surface state of CDs, which can impact the band gap, or the influence of diverse emission sites on various size nanoparticles in the sample. Note that when excited at 380 nm, the PL intensity of CDs achieves its maximum at 466 nm. Therefore, 380 nm are chosen as excitation wavelengths and emissions at 466 nm are collected for further study.

**Stability analysis**

As we all know, the application of the CDs requires optical stability as well as structural stability against the ionic strength. Thus, the effects of the ionic strength on the fluorescence intensity of the product and its relevance to sensing applications were evaluated. In Fig. 4a, the fluorescence intensity changes only slightly, even under the condition of high ionic strength, indicating that these CDs are stable. In addition, we also investigated the fluorescence intensity with a pH value of 1 – 11. In Fig. 4b, the fluorescence intensity first increased and then decreased with the increase of pH value. In addition, the fluorescence intensity of CDs in acidic media is stronger than that in alkaline media, reaching its maximum value near pH = 3. That is to say, the CDs illustrate pH dependence, which is related to the protonation/non-protonation of the surface groups. To further evaluate the stability, the influence of anions was investigated. PL is minimally observed upon the addition of 10$^{-4}$ M anions. In Fig. 4c, there is no disturbance to the PL intensity of the CDs, further suggesting the high stability. Such CDs with strong and stable PL will offer great potential for widespread applications, like sensors and biomedical imaging.

**The application of CDs in Fe$^{3+}$ detection**

The effect of different metal ions quenching on the fluorescence properties of CDs shows that the as-prepared CDs is a promising metal ion fluorescent probe. In order to evaluate the selectivity, we studied PL in the presence of the usual metal ions under identical conditions. In Fig. 4d, the PL intensity ratio $[F/F_0]$ is the lowest, while other metal ions only show slight variations, which turned out that the presence of Fe$^{3+}$ will lead to a significant reduction in the fluorescence intensity compared with other metal ions. This indicates that the CDs show high selectivity for Fe$^{3+}$. The synthetic CDs have a variety of groups on the surface, for example, C-O-C, C=O, C-H, and C-OH, which is apparent from XPS, FTIR, and Raman results. They can react with Fe$^{3+}$ to form complexes, effectively preventing the hydrolysis of Fe$^{3+}$ in the solution, and eventually leading to the fluorescence quenching of CDs. They may lead to a string of emissive traps between the $\pi$ and $\pi^*$ states of C=C. The surface energy trap can control the emission when a certain excitation wavelength irradiates the CDs. With a change of the excitation wavelength, another corresponding surface state emissive trap will be dominant. Hence, we regard that surface-bound Fe$^{3+}$ captures the photo-excited electrons of CDs, resulting in PL quenching processes. That is to say, the surface of the CDs will change after adding Fe$^{3+}$, leading to poor luminescence efficiency. This is consistent with the reported work.

The sensitivity of Fe$^{3+}$ at different concentrations within the scope of 0 – 1000 μM was studied. Figure 5a reveals that the PL emission intensity at 466 nm is highly sensitive to Fe$^{3+}$ and decreases with the increment of the Fe$^{3+}$ concentration without displacement, indicating that fluorescence quenching is concerned with the concentration of Fe$^{3+}$. Stern-Volmer (SV) relation has been applied to study the mechanism of fluorescence quenching. The fluorescence quenching data accord with Stern-Volmer equation.
\[ F_0/F = 1 + K_{sv}[Q], \]  

where, \( F_0 \) and \( F \) are the PL strength of CDs at 466 nm without and with Fe\(^{3+}\), respectively. \( K_{sv} \) is the Stern-Volmer quenching constant, and \([Q]\) is the concentration of Fe\(^{3+}\). Figure 5b shows the relation between \([F_0/F - 1]\) and the concentration of Fe\(^{3+}\). The Stern-Volmer diagram shows two different slopes over the whole concentration range, which does not conform to the traditional Stern-Volmer linear equation. It suggests that both dynamic and static quenching processes exist in the sensing system.\(^{78-81}\) The correlation coefficients \( (R^2) \) of Fe\(^{3+}\) are 0.9951 and 0.9969 in the ranges of 0 – 100 and 100 – 1000 \( \mu \)M, respectively. The linear relationship of the fluorescent quenching and the Fe\(^{3+}\) concentration indicate that these CDs can selectively and quantitatively detect Fe\(^{3+}\). The detection limit is estimated to be at 0.1 \( \mu \)M, which is less than the highest content (0.3 mg/L, which corresponds to 5.4 \( \mu \)M) of Fe\(^{3+}\) in drinking water allowed by the US Environmental Protection Agency (USEPA).\(^{82}\) This suggests that this sensing system has outstanding sensitivity and a huge potential for Fe\(^{3+}\) detection.

**Application of CDs in fluorescent ink**

Due to its good fluorescence, outstanding stability and water-solubility, the as-prepared N-doped CDs can be used as fluorescence ink. As shown in Fig. 6b, the clear fluorescent characters “Carbon Nanodots” engraved on the filter paper using the as-prepared CDs fluorescent ink can be seen under 356 nm ultraviolet light, but you can not see them in the daylight (Fig. 6a). That is to say, the use of CDs fluorescent ink can obtain high quality and high stability fluorescent graphics and text characters. We believe that CDs fluorescent ink could be used for information encryption and storage in the near future.

**Conclusions**

In conclusion, an effective strategy has been demonstrated here for synthesizing N-doped fluorescent CDs with blue fluorescence by a hydrothermal treatment of fermented rice. What is more, Fe\(^{3+}\) can quench the PL of CDs. Based on this, we developed a PL detection system for Fe\(^{3+}\) in aqueous solutions. This study offers a simple and cheap method for green production of CDs for sensing Fe\(^{3+}\) with several merits. First, the method presented here is easily conducted. Second, no further chemical modification of CDs is needed, which illustrates its simplicity and low cost. Last, these CDs can act as a successful sensing platform toward Fe\(^{3+}\). Along with this cost-effective protocol of synthesis and satisfactory sensing properties, we expect that these novel CDs have potential applications in biological and environmental studies.

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