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Research Article

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Carbon Dots from Willow Leaves for Fe^{3+} Detection: A Comprehensive Undergraduate Experiment in Analytical Chemistry

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Abstract

The nitrogen-doped carbon dots (N-CDs) were prepared by one-step hydrothermal method at 200 ℃ for 6 h using willow leaves as carbon source and urea as nitrogen source. The structure of the as-prepared N-CDs was characterized by transmission electron microscopy (TEM), Fourier transform infrared spectrum (FT-IR), X-ray photoelectron spectrum (XPS) and UV-vis absorption spectrum. The maximum fluorescence excitation and emission wavelengths of N-CDs were located at 380 nm and 453 nm, respectively. The fluorescence quantum yield was calculated to be 8.6%. Based on the fluorescence quenching effect of N-CDs toward Fe^{3+}, a new and rapid detection method for Fe^{3+} was established. The results showed that using N-CDs as fluorescent labeling, the linear relationship for Fe^{3+} detection was obtained in the range of 75 to 300 μM, and the detection limit reached 3.19 μM. This comprehensive experiment covers the preparation process, characterization methods, experimental detection and other aspects of nanomaterials, which can stimulate the students' enthusiasm to explore and innovate, cultivate the students' ability to analyze and solve problems independently, and promote the integration of students' theoretical knowledge and practical ability. It not only exercises students' comprehensive ability to use knowledge and experimental skills, but also helps to broaden students' academic vision.

Keywords: Willow Leaf, Carbon Dots, Chemical Education, Comprehensive Experiment, Fe^{3+}

1. Introduction

Comprehensive experiment is usually a systematic project that can organically integrate the relatively scattered knowledge in the experimental course. It has certain integrity, relevance and interdisciplinary nature. It is also a method to adapt to the new needs of national development and the new trend of the world's scientific and technological frontier development, and cultivate innovative, applied and compound high-quality talents [1]. Comprehensive chemical experiment is a compulsory experimental course for undergraduate students in chemistry, chemical engineering, materials, environment, food and pharmacy, and plays a vital role in training students to master modern analytical technology [2]. However, in the current stage of chemical experiment teaching, the construction, use steps and detection principles of instruments are mainly taught, and the basic elements of chemistry and the preparation and application of materials in the forefront of discipline development are often neglected, which makes the content of chemical experiment teaching incomplete and fails to achieve the organic combination of basic experiments and comprehensive experiments. In addition, the experimental items involved in the current experimental teaching are single in method and isolated in content. Each experimental item is often taught independently and lacks relevance, which leads to students' low learning enthusiasm, weak grasp of basic knowledge, mechanical experiments according to the experimental steps, and lack of solutions to the problems arising in the experimental process [3]. The above problems seriously restrict the improvement of students' scientific research thinking and innovative practice ability, and are not conducive to the cultivation of students' active learning ability, knowledge application ability and innovation ability.

Based on the above shortcomings, we should attach importance to experimental teaching, combine theory with practice, and improve students' learning interest. Therefore, designing comprehensive experiments is the key to solve the problem. From the latest scientific research achievements, a comprehensive chemical experiment project "Willow Leaves-Derived Carbon Dots and Their Application in Fe^{3+} Detection" was designed. It has been practiced and explored in the undergraduate chemical experiment teaching course. Through the successful implementation of this comprehensive experimental project, on the one hand, it can organically integrate the academic and scientific frontier with the basic skills experiment, which is research-oriented and application-oriented.

2. Experimental Design

2.1. Experimental Background

Carbon dots (CDs) are a new type of carbon-based nanomaterials with size below 10 nm. The main elements of CDs contain carbon, hydrogen, oxygen and nitrogen [4]. CDs have gained considerable interest due to their distinctive properties, such as simple fabrication, robust chemical inertness, low toxicity, tunable photoluminescence (PL), and excellent biocompatibility [5]. The synthesis methods of CDs are mainly divided into two types, namely "top-down" and "bottom-up" [6]. "Bottom-up" method such as hydrothermal synthesis is the most commonly used method at present, with the advantages of simple operation and high quantum yield [7]. Hydrothermal synthesis is the most widely used method in teaching and practical research because it's easy to operate and has low requirements for equipment. The previous literatures showed that the partial modification of the surface of CDs, such as doping with N, S, and P can effectively improve the optical properties of CDs [8, 9].

Carbon source is an important component for the preparation of carbon dots [10]. Recently, biomass has become the most popular carbon source due to their advantages of easy access, low cost, green environmental protection, and rich resources [11]. The common biomass-based carbon sources include vegetables, fruits, grass, leaves and even domestic garbage [12]. The biomass-derived carbon dots are applied in many fields such as biomedicine, sensing, photocatalysis, fingerprint detection, etc [13]. The previous studies demonstrated that carbon dots that prepared from mustard seeds, cumin, garlic and black rice can be used for ion detection, temperature sensing and biological imaging [14]. The carbon dots that prepared from kiwifruit juice can be used to determine the content of Fe^{3+} in honeysuckle, and the carbon dots prepared from pomegranate seeds can be used to determine the content of tiopronin in human serum samples [15].

In this comprehensive experiment, the nitrogen-doped biomass-derived carbon dots (N-CDs) were prepared by one-step hydrothermal method with willow leaves as carbon source and urea as nitrogen source. The fluorescence quenching effect of various ions on N-CDs was preliminarily investigated, and its fluorescence was demonstrated to have selective quenching effect on Fe^{3+}. Subsequently, the quenching degree of the fluorescence of N-CDs by different concentrations of Fe^{3+} was measured. Based on these results, the linear relationship between fluorescence intensity and concentration, and the detection limit was obtained. Through the study of this experiment, firstly, students can exercise their experimental operation ability and master the skills of using ultraviolet absorption and fluorescence spectrometer. Secondly, students can strengthen their understanding of the detection limit of ions and master relevant calculation methods through fluorescence analysis and detection. Thirdly, it could exercise students' ability to use various software to process data. Finally, it will broaden students' horizons of knowledge, and cultivate students' interest in experimental research.

2.2. Teaching Design

The chemical comprehensive experiment of detecting Fe^{3+} using biomass-based carbon dots can be divided into three stages.

(1) Preview before class. Before the experiment begins, students should make preparations in advance, search for relevant documents through the library database, understand the concept of biomass-based carbon dots, preparation method, the basic working principle of UV-visible spectrophotometer and fluorescence spectrophotometer, and the principle of Fe^{3+} detection using carbon dots. These processes not only exercise students' independent learning ability, but also guide students to use the time outside the classroom to consult relevant documents, which make students realize the difference between comprehensive chemistry experiment and other experiments.

(2) Classroom experiment. Firstly, before the experiment, the teacher asked questions about the knowledge that students learned from the literature before class. Meanwhile, according to the students' feedback, the teacher made a brief supplement, and taught the experimental steps and precautions of hydrothermal method to prepare biomass-based carbon dots. This process can inspect the effect of students' autonomous learning and deepen students' understanding of knowledge. Secondly, students use willow leaves as raw materials to prepare carbon dots by hydrothermal method, which cultivates students' awareness of safety, green environmental protection and innovation. This process can make students realize that chemistry is a science close to life, which is oriented to national needs and solves practical problems.

(3) After the experiment. Students should learn relevant data processing software (such as Origin and Excel software) by themselves, and write the experiment paper. In this process, it not only exercises students' data processing ability, but also strengthens the cultivation of students' scientific research ability and further improves students' comprehensive ability.

3. Purpose of the Experiment

(1) Learn the preparation method of N-CDs.

(2) Master the use method and spectrum analysis of UV-visible spectrophotometer, infrared spectrometer and fluorescence spectrometer.

(3) Master the linear detection of substances and the calculation method of detection limit.

(4) Learn to use various software to process and analyze experimental data and draw relevant conclusions.

(5) Strengthen and consolidate the experimental operation ability and broaden students' vision.

4. Experimental Materials and Methods

4.1. Materials and Instruments

Ethylenediamine, urea, thiourea, ammonia, DMF, ferric trichloride, zinc chloride, calcium chloride, stannous chloride, magnesium chloride, cobalt dichloride, mercuric chloride, cupric chloride dihydrate, manganese chloride, lead chloride, ammonium chloride, glacial acetic acid, and sodium acetate were purchased from Inokey Chemical Reagents Co., Ltd. FT-IR spectrum was collected by a NICOLET6700 spectrometer. TEM images were detected through a JEM-3010 instrument (JEOL). The absorption spectrum was measured on a UV-vis spectrophotometer (Shimadzu). The fluorescence spectrum was collected on a FluoroMax-4 spectrofluorometer (JOBIN YVON Technology). XPS was performed using an SKL-12 spectrometer modified with a VGCLAM 4 multichannel hemispherical analyzer.

4.2. Experimental Method

4.2.1. Preparation of N-CDs

The fluorescence properties of N-CDs are closely related to the preparation conditions. The preparation conditions include reaction temperature, reaction time, nitrogen source and the feed ratio of willow leaves and nitrogen source. The influence of the above related factors on the fluorescence intensity of N-CDs is discussed through the control variable method, so as to obtain N-CDs with higher fluorescence intensity and better performance.

(1) Reaction temperature. Firstly, 9 g of fresh willow leaves was added to 24 mL of purified water. After stirring thoroughly with the juicer, the crude willow leaf juice was obtained. The juice was then centrifuged to remove the precipitate, and the supernatant was divided into three groups. 2 mL of ethylenediamine was then added to each group. After ultrasonic treatment for 5 min, each solution was transformed into Teflon-lined autoclave. The hydrothermal temperature was set at 160 °C, 180 °C, and 200 °C respectively. After 8 h, the resulting solution was centrifuged to remove solid precipitation. The obtained solution was then dialyzed against deionized (DI) water for 48 h, and the fluorescence intensity of each product solution was measured and compared.

(2) Heating time. The willow leaf juice was prepared according to the same method as above. It was then divided into five groups, and each group was added with 2 mL of ethylenediamine. After ultrasonic treatment for 5 min, each solution was transformed into Teflon-lined autoclave. The hydrothermal temperature was set at 200 °C and each solution was stayed for 2, 4, 6, 8, and 10 h respectively. The resulting solution was then centrifuged to remove solid precipitation. The obtained solution was dialyzed against deionized (DI) water for 48 h, and the fluorescence intensity of each product solution was measured and compared.

(3) Nitrogen source. The willow leaf juice was prepared according to the same method as above. It was then divided into five groups, and each group was added with 0.03 mol ethylenediamine, urea, thiourea, DMF, and ammonia respectively. After ultrasonic treatment for 5 min, each solution was transformed into Teflon-lined autoclave. After heating at 200 °C for 6 h, the resulting solution was centrifuged to remove solid precipitation. The obtained solution was then dialyzed against deionized (DI) water for 48 h, and the fluorescence intensity of each product solution was measured and compared.

(4) Ratio of willow leaves and nitrogen sources. The willow leaf juice was prepared according to the same method as above. It was then divided into four groups, and each group was added with 0.3 g, 0.6 g, 0.9 g and 1.2 g of urea respectively. After heating at 200 °C for 6 h, the resulting solution was centrifuged to remove solid precipitation. The obtained solution was then dialyzed against deionized (DI) water for 48 h, and the fluorescence intensity of each product solution was measured and compared.

4.2.2. Fluorescence Intensity of N-CDs at Different Test Conditions

In addition to the preparation conditions that will affect the fluorescence intensity of N-CDs, other relevant test conditions will also affect its fluorescence intensity, such as the excitation wavelength, the pH value of buffer solution, and the concentration of N-CDs.

(1) Excitation wavelengths. The as-prepared N-CDs solution was subjected to fluorescence detection at a series of different excitation wavelengths (320 nm-420 nm). The excitation wavelength that induced the maximum fluorescence intensity was obtained.

(2) Buffer solution with different pH values. The as-prepared N-CDs solution was subjected to fluorescence detection at a series of different pH value (1, 3, 5, 7, 9 and 11). The pH value of buffer solution that induced the maximum fluorescence intensity was obtained.

(3) Concentration of N-CDs solution. The as-prepared N-CDs solution was subjected to fluorescence detection at a series of different concentrations. The concentration of N-CDs that induced the maximum fluorescence intensity was obtained.

4.2.3. Characterization

The morphology of N-CDs was observed by transmission electron microscopy (TEM). The chemical composition and functional groups of N-CDs were analyzed by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FT-IR). The photophyscial properties of N-CDs were studied by UV-Vis spectrophotometer and fluorescence spectrometer.

4.2.4. Calculation of Fluorescence Quantum Yield

The fluorescence quantum yield of CDs was measured with quinine sulfate as the standard (quantum yield is 54.2%). Specifically, the absorbance and fluorescence integral area of the sample and quinine sulfate solution at 350 nm were determined. The fluorescence quantum yield of CDs was calculated according to the following formula.

$$\varphi_f = \varphi_f^R \frac{I_f}{I_f^R} \frac{A^R}{A} \left(\frac{n}{n^R}\right)^2$$

Where, φ is fluorescence quantum yield, A is absorbance, I is fluorescence integral area, and n is solvent refractive index.

4.2.5. Fluorescence Detection of Fe3+ by N-CDs

In order to obtain the selectivity of N-CDs, the aqueous solutions of $FeCl_3$, $ZnCl_2$, $CaCl_2$, $SnCl_2$, $MgCl_2$, $CoCl_2$, $HgCl_2$, $CuCl_2$, $MnCl_2$, $PbCl_2$, and NH_4Cl with cation concentration of 10 mM were prepared. To a 3 mL test tube, 200 μL N-CDs solution, 50 μL of the prepared cation solution, and 2.75 mL of buffer solution with pH 5 were added. The fluorescence spectrum of each solution was recorded, and the fluorescence quenching effect of each ion toward N-CDs was determined.

4.2.6. Detection Limit of N-CDs for Fe^{3+}

Based on the fluorescence quenching effect of different concentrations of Fe^{3+} on N-CDs, the detection range and detection limit of N-CDs toward Fe^{3+} can be obtained according to the calculation formula (LOD=3 σ/k). σ is the blank standard deviation value obtained by repeatedly measuring the fluorescence of N-CDs solution for 14 times using fluorescence spectrum. k is the slope of the linear relationship between F_0-F and concentration of Fe^{3+}.

5. Results and Discussion

5.1. N-CDs Preparation

The reaction temperature and time have a direct impact on the fluorescence intensity of N-CDs. Under different reaction temperature and time, the fluorescence intensity of N-CDs has great differences. As shown in Fig. 1a, the fluorescence intensity of N-CDs gradually increased with the reaction temperature. When the hydrothermal temperature was set as 200 °C, the fluorescence intensity of N-CDs was the strongest. When the reaction time is 2 h, the fluorescence intensity of N-CDs is the lowest. Besides, when the reaction time was 6 h, the fluorescence intensity of the prepared N-CDs solution was the highest. With the further extension of the reaction time, the fluorescence intensity gradually decreased (Fig. 1b).

The nitrogen-containing compounds such as urea, ethylenediamine and thiourea were usually used as surface modifier to fabricate the nitrogen-doped carbon dots. During the synthesis of N-CDs, the influence of nitrogen sources (ethylenediamine, urea, ammonia, thiourea and DMF) on the fluorescence of N-CDs was investigated. As shown in Fig. 1c, the N-CDs that prepared with urea as the surface modifier had the strongest fluorescence. Therefore, urea was chosen as the surface modifier for nitrogen doping in subsequent experiments.

During the experiment, the mass ratio of willow leaves and urea also has a certain influence on the fluorescence intensity of N-CDs, which determines the nitrogen content in N-CDs, thus further affecting the fluorescence intensity of products. Therefore, this experiment adopts different proportions to carry out the experiment in order to find the best feeding mass ratio and obtain the N-CDs solution with the highest fluorescence intensity. As shown in Fig. 1d, when the mass of willow leaves and urea was adjusted to 5:1, the fluorescence intensity of N-CDs was the highest.

According to the above optimization experiment results, N-CDs had the highest fluorescence intensity under the following condition: urea as the nitrogen source, mass ratio of willow leaves to urea 5:1, hydrothermal temperature 200 °C, and reaction time 6 h.

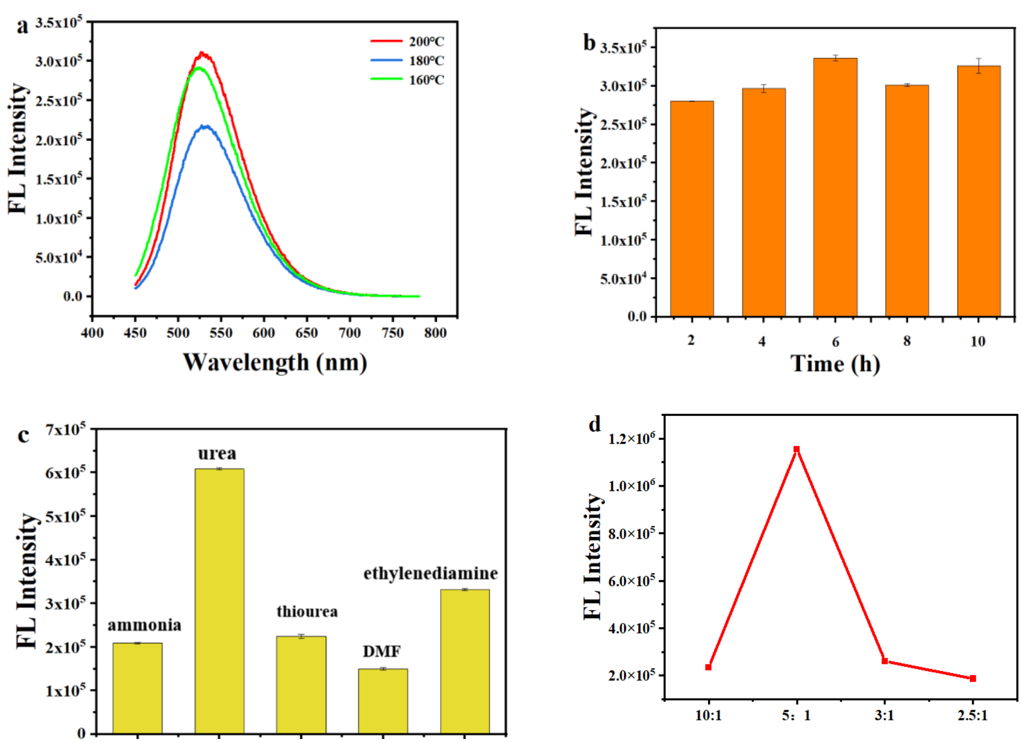

Figure 1: (a) Effect of reaction temperature on the fluorescence intensity of N-CDs; (b) Effect of reaction time on the fluorescence intensity of N-CDs; (c) Effect of nitrogen sources on the fluorescence intensity of N-CDs; (d) Effect of the mass ratio of willow leaves to nitrogen source on the fluorescence intensity of N-CDs.

5.2. Fluorescence Intensity of N-CDs Under the Test Conditions

N-CDs showed the excitation-dependent emission properties, indicating that the excited state of CDs was adjustable, which was probably related to the uneven particle size distribution of CDs, different functional groups on the surface and surface emission defects. As shown in Fig. 2a, the fluorescence intensity of N-CDs reached the maximum when the excitation wavelength was set as 380 nm.

pH value also affects the fluorescence of carbon dots. Therefore, the fluorescence spectrum of N-CDs at different pH was measured. As shown in Fig. 2b, the fluorescence intensity of N-CDs under acidic conditions was stronger than that under alkaline conditions. When the pH value of the buffer solution was 5, the N-CDs exhibited the strongest fluorescence intensity.

The concentration of N-CDs had the significant effect on their fluorescence intensity. As shown in Fig. 2c, the fluorescence intensity of N-CDs first increased and then decreased with their concentration. When the concentration of N-CDs solution was set as 0.71 mg/mL, they had the highest fluorescence intensity.

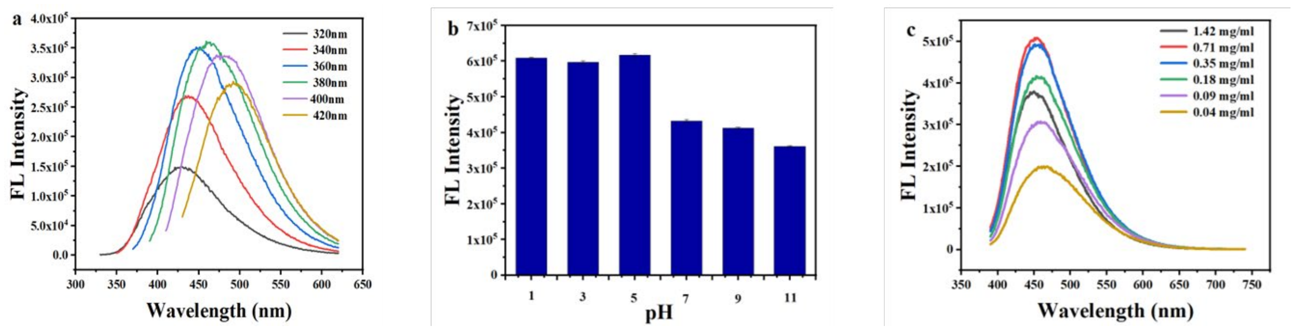

Figure 2: (a) Fluorescence spectrum of N-CDs at different excitation wavelengths; (b) Effect of pH value on the fluorescence intensity of N-CDs; (c) Effect of N-CDs concentration on their fluorescence intensity.

5.3. Characterization of N-CDs

The particle size of N-CDs was characterized by transmission electron microscopy (TEM). As shown in Fig. 3a, N-CDs were spherical and well dispersed in water with average size about 5 nm. The FT-IR spectrum of N-CDs (Fig. 3b) showed the characteristic peak at 3423 cm^{-1}, which could be assigned to the stretching vibration of -OH or -NH-. The signals at 1632, 1405, and 1124 cm-1 were attributed to the stretching vibration of C=O, C-N, and C-O bond respectively. Thus, the surface of N-CDs contained -OH, -NH-, C-N, C-O, and other functional groups.

The photophysical properties of N-CDs were evaluated through the absorption and fluorescence spectrum. As shown in Fig. 3c, the absorption spectrum of both N-CDs and willow leaf juice displayed the strong peak around 230 nm. Moreover, N-CDs showed an obvious absorption peak at 300 nm, which was mainly ascribed to the π-π* transition in the sp2 region of N-CDs. The aqueous solution of N-CDs was dark brown under the sunlight, and showed extremely strong blue luminescence under irradiation of 365 nm UV lamp (insert of Fig. 3d). Under excitation of 380 nm, N-CDs exhibited the maximum fluorescence intensity at 453 nm. Using quinine sulfate (quantum yield of 54%) as the standard reference, the fluorescence quantum yield of N-CDs was calculated to be 8.6%.

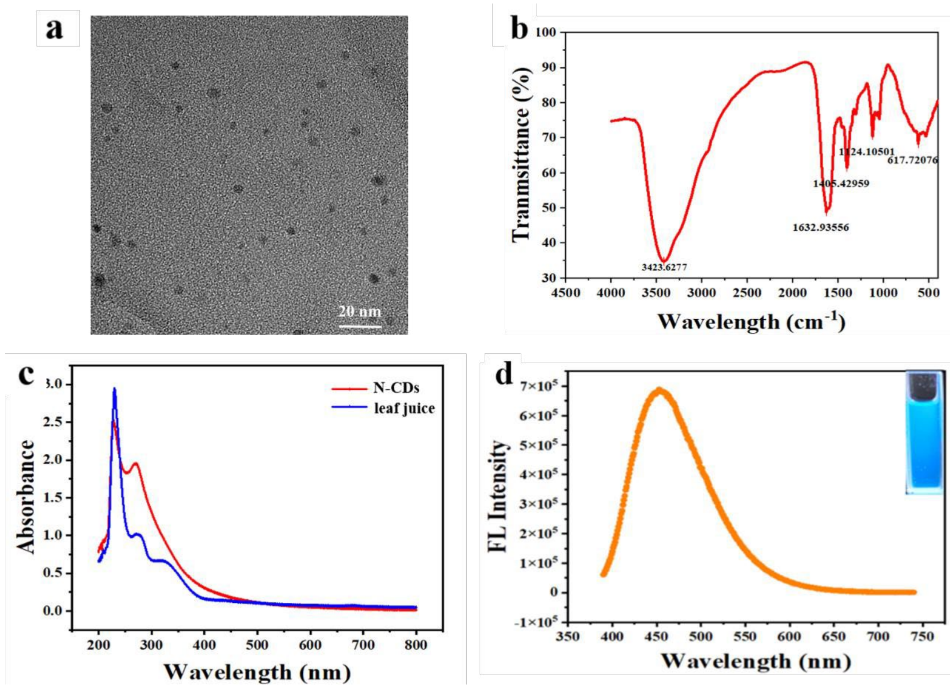

Figure 3: (a) TEM image of N-CDs; (b) FT-IR spectrum of N-CDs; (c) UV-Vis absorption spectrum of N-CDs aqueous solution and willow leaf juice; (d) Fluorescence spectrum of N-CDs (the insert shows the fluorescence of N-CDs under 365 nm laser irradiation).

The element composition of N-CDs was characterized by XPS spectrum. As shown in Fig. 4a, three characteristic peaks were observed at 293.12, 396.45 and 534.27 eV, assigned to C1s, N1s and O1s respectively. A high-resolution XPS spectrum of C 1s (Fig. 4b) presented four peaks at 284.3, 285.0, 285.6 and 288.2eV, which were ascribed to C=C, C-C, C-N and C=O respectively.

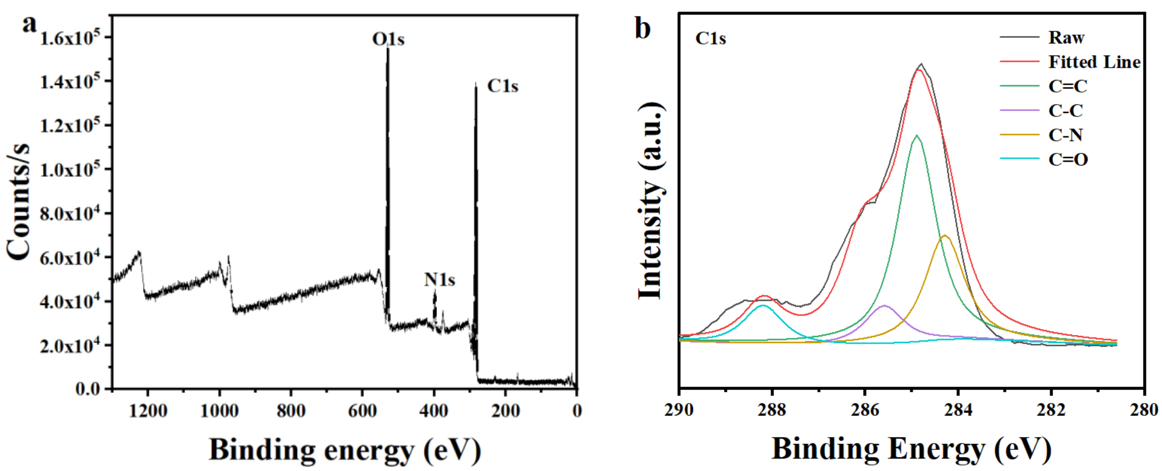

Figure 4: (a) XPS spectrum of N-CDs; (b) High resolution C1s spectra of N-CDs.

5.4. Fluorescence Detection of Fe^{3+} by N-CDs

The fluorescence spectrum of N-CDs was recorded upon the addition of 11 different ions (Pb^{2+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, Zn^{2+}, NH^{4+}, and Sn^{2+}). As shown in Fig. 5a, Fe^{3+} had an obvious quenching effect on the fluorescence intensity of N-CDs, while the quenching effect of other ions was not significant and can be ignored. Thus, N-CDs showed the high selectivity for Fe^{3+} and can be used as a fluorescence sensing probe. Moreover, the quenching effect of Fe^{3+} at different concentrations was investigated. As shown in Fig. 5b, the fluorescence intensity of N-CDs decreased gradually with the concentration of Fe^{3+} from 0 to 330 μM. Accordingly, the emission of N-CDs changed from bright blue to dark under UV irradiation (Fig. 5c). Moreover, the fluorescence changes of N-CDs (F_0-F) and Fe^{3+} concentration showed a good linear relationship in the range of 100-300 μM (Fig. 5d), with the correlation coefficient (R^2) of 0.99639.

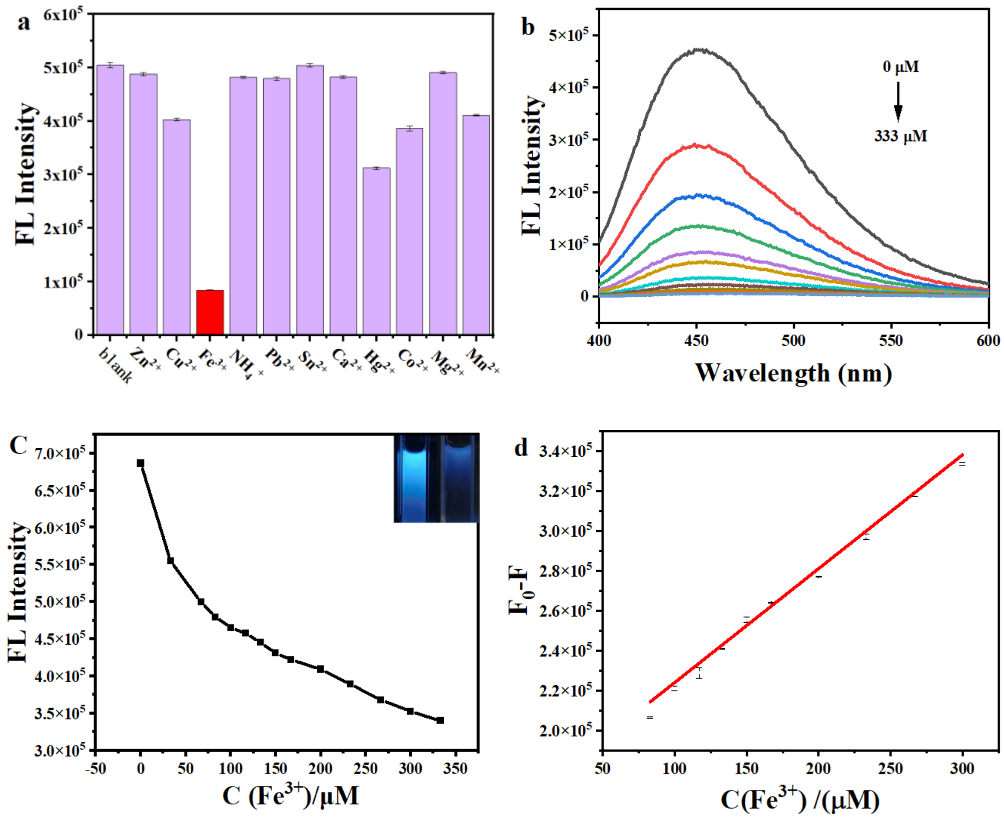

Figure 5: (a) Ion selectivity of N-CDs; (b) Fluorescence spectrum of N-CDs upon the addition of Fe^{3+} (c) Effect of Fe^{3+} concentration on the fluorescence intensity of N-CDs; (d) Linear relationship between F_0-F and Fe^{3+} concentration.

5.5. Calculation of Detection Limit

The detection limit of Fe^{3+} was calculated according to the following formula.

$$LOD = \frac{3SD}{S}$$

Where, LOD is the detection limit, SD is the standard deviation of the blank sample, and S is the slope of the linear relationship.

Based on the data in Fig. 5d, we can get the linear formula of F_0-F=167017.43+570.75[Fe^{3+}]. Through the fluorescence detection of blank samples, the standard deviation was calculated to be 607.17143. Thus, the detection limit was valued as 3.19 µM according to the data of SD and S.

6. Experimental Effect Evaluation

(1) Submission of experimental results. Each student submits an experiment report, which requires the language to be concise and clear. The experimental results should be presented in the form of charts and theoretical analysis. Teachers should organize students to exchange and discuss the experimental report carefully, summarize the gains and shortcomings of the experiment, and propose improvement methods. Meanwhile, the instructor can introduce the skills of data processing, origin drawing and curve fitting, word document editing, etc. in a targeted way to improve students' ability in this field.

(2) Performance evaluation. The students' comprehensive experimental results consist of three parts: 50% of the experimental report, 40% of the classroom experimental performance, and 10% of the attendance.

(3) Through the training of this comprehensive experiment, students' enthusiasm and initiative in learning professional knowledge can be improved. Besides, it can exercise and improve students' experimental skills, ability to analyze and solve problems, and innovation ability. Finally, this kind of open experimental teaching can also cultivate the spirit of teamwork, exercise the ability of organization and management, and develop a rigorous academic attitude.

(4) During the whole teaching process, the teacher guides the students to actively think about the principle and significance of each experimental step, and correct the problems found in time. For students, they should not only follow the experimental operation steps, but also learn to think, find problems, solve problems and draw conclusions in each experimental operation. Compared with a single experimental project, the implementation of this comprehensive experiments can stimulate students' innovation and exploration consciousness, and significantly improve their comprehensive quality.

7. Conclusion

N-CDs were prepared by one-step hydrothermal method with willow leaves as carbon source and urea as surface modifier. Through the optimization of experimental conditions, the best preparation methods of N-CDs were finally determined, that is, the mass ratio of willow leaves to urea was 5:1, the hydrothermal temperature was 200 ℃, and the reaction time was 6 h. In addition, the fluorescence properties of the as-prepared N-CDs were tested, and the concentration of N-CDs at the maximum fluorescence intensity was confirmed through experimental optimization. The fluorescence of N-CDs showed selective quenching effect toward Fe^{3+}, with the detection limit as low as 3.19 μM, which is expected to be applied to the detection of Fe^{3+} in real water samples and human body.

This experiment is systematic, comprehensive and research-oriented. Through the successful implementation of this experimental project, students can deepen their understanding of UV-Vis absorption spectrometry and fluorescence spectrometry, and exercise their hands-on operation skills. Through the enrichment and renewal of analytical chemistry experiment projects, it is bound to stimulate students' learning enthusiasm, cultivate students' scientific research innovation awareness and comprehensive ability, and comprehensively improve the teaching quality of university analytical chemistry experiment courses.

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