

# Preparation of boron doped fluorescent carbon dots and detection

Dong Lin, Chen Qian

College of Chemical Engineering, University of Science and Technology Liaoning, Anshan, 114000, China

**Abstract:** A kind of blue fluorescent carbon dots (B-CDs) was synthesized by hydrothermal synthesis using chitosan as carbon source, boric acid as dopant reagent and water as solvent. The spherical carbon dots with an average particle size of 1.84 nm and lattice spacing of 0.21 nm were characterized by HRTEM and XRD. Boron was successfully doped into B-CDs by FTIR and XPS analysis. Based on the dynamic quenching and photoinduced electron transfer mechanism, the sensitive detection of  $Hg^{2+}$  was realized. The linear detection range was 0-67  $\mu M$ , and the detection limit was 0.233  $\mu M$ . The carbon point was applied to the detection of water samples and cosmetics samples with recoveries of 98.6-102.1% and 95.6-103.2%, respectively.

**Keywords:** Carbon point; Fluorescence detection; Quenching mechanism

## 1. Introduction

As we all know, mercury pollution caused by human activities mainly comes from waste water discharged by chlor-alkali, plastics, batteries, electronics and other industries and waste medical equipment. It is estimated that the total amount of mercury released directly into water bodies through human activities was approximately 16,000 tons during 1970 and 1979 [1]. The total amount of mercury released into the atmosphere amounted to about 100,000 tons. The total amount of mercury released to the soil is about 100,000 tons, and the mercury released to the atmosphere and soil also returns to the water body with the water cycle. In the natural background, mercury is distributed in the atmosphere, soil, and water, and the movement of mercury also occurs between land, water, and sky. Atmospheric mercury in gaseous and granular form is dispersed by the wind, and some falls to the ground or water through wet or dry deposition. Mercury in the soil can evaporate into the atmosphere, or it can be washed into surface waters and leach into groundwater through precipitation. Some of the mercury in surface water evaporates into the atmosphere, and most of it settles into the soil. Mercury in soil, regardless of its form, can be converted directly or indirectly to methylmercury or dimethylmercury by microorganisms. Methylmercury is decomposed into methylmercury under acidic conditions. Methylmercury is soluble in water, so it returns from the soil to the water. Methylmercury ingested by aquatic organisms accumulates in the body and becomes increasingly abundant through the food chain. Mercury contamination of fish in bodies of water, with concentrations of methylmercury 10,000 times higher than in water, can not only threaten fish, but even humans through the food chain. So it makes sense to use scientific methods to detect mercury.

The commonly used methods for the detection of heavy metal ions include mass spectrometry [2], electrochemical detection [3], X-ray [4] and fluorescence spectrometry [5]. However, due to economic factors, most of these methods are difficult to be widely used. As we all know, CDs prepared by carbon points as carbonuclear biomaterials with  $sp^2$  structure domain and surface functional groups have good biocompatibility, stable fluorescence properties, non-toxicity and other characteristics, and have broad application prospects in this field [6]. Chitosan, a linear polysaccharide linked by B-glucoside bond, is a widely distributed product of chitin deacetylation in nature. Chitosan is widely used in drug synthesis due to its easy availability and good biocompatibility. Chemical analysis and other fields [7-10]. In addition, chitosan-based carbon points are mainly used for biosensors and bioimaging. Song [11] et al. prepared water-soluble and reducible carbon quantum dots (CQDs) by hydrothermal carbonization of chitosan. As reducing agent and stabilizer, the prepared CQDs were further used to synthesize gold nanoparticles (AuNP). The synthesis process is carried out in an absolutely "green" aqueous solution. In addition, CQDs/AuNPs composites were used to detect iodine ions by colorimetry. In this chapter, a blue-green fluorescent carbon nanoparticle (B-CDs) was prepared using chitosan as raw

material, and  $\text{Hg}^{2+}$  ions were detected efficiently. B-CDs was synthesized by one-step hydrothermal method, which is simple, cheap, environmentally friendly and suitable for large-scale production. In addition, due to the combined action of electron transfer and aggregation, the fluorescence intensity of carbon dots decreases rapidly, and even fluorescence quenching occurs with heavy metal ions. Through the fluorescence quenching effect, the linear analysis of the concentration of heavy metal ions and the fluorescence intensity of this carbon point shows that this carbon point can detect  $\text{Hg}^{2+}$  in wastewater. Therefore, the carbon point is expected to be widely used in sewage detection and treatment.

## 2. Materials and Methods

### 2.1 Reagents and Instruments

Laboratory-grade thiourea, boric acid, anhydrous ethanol, sulfuric acid, quinine sulfate, sodium chloride, zinc chloride, ammonium chloride, copper nitrate, silver nitrate, cobalt sulfate, manganese sulfate, sodium sulfate, sodium hydroxide, sodium carbonate, and phosphoric acid were all purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd. Ethylenediamine tetraacetic acid disodium salt, potassium chloride, barium chloride, cadmium chloride, nickel chloride, aluminum chloride, potassium dihydrogen phosphate, sodium sulfide, sodium dihydrogen phosphate, calcium sulfate, iron chloride, sodium nitrite, sodium acetate, ferrous sulfate, mercury nitrate, and sodium carbonate were sourced from Shenyang Reagent Factory. Chitosan was obtained from Aladdin Reagent Co., Ltd. Magnesium nitrate, sodium fluoride, sodium dihydrogen phosphate, and chromium chloride were acquired from Tianjin Bodhi Chemical Co., Ltd. The water used in the experiments was ultrapure water with a resistivity of over  $18.3 \text{ M}\Omega\text{cm}^{-1}$ .

The experimental equipment used in this study included 50 mL and 200 mL high-temperature and high-pressure reaction vessels (Xi'an Taikang Instrument), a 202-0 electric constant-temperature drying oven (Shanghai Hunan Scientific Instrument Co., Ltd.), an 88-1 constant-temperature magnetic stirrer (Changzhou Guohua Electric Appliance), a PHS-25 LecipH pH meter produced by Shanghai Yidian Scientific Instruments, a rotary evaporator (RE 2000E) from Shanghai Hujia Instruments and Equipment, a freeze dryer (FD-1A-50) from Shanghai Xinweng Scientific Instrument Co., Ltd., a KQ-300DE ultrasonic cleaner from Kunshan Ultrasonic Instruments, a JEM2100F high-resolution transmission electron microscope produced by Philips-FEI in the Netherlands, an X-ray diffractometer (X-pert Powder, PANalytical, Netherlands), a Fourier-transform infrared spectrometer (EQUINOX55, Bruker, Germany), a UV-visible spectrophotometer (L6S, Shanghai Yidian Analytical Instrument Co., Ltd.), a fluorescence spectrophotometer (FS5, Edinburgh Instruments, UK), an ESCALAB 250 Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific), a HORIBA FluoroMax-4 fluorescence spectrometer from Horiba Scientific in France, a Zeta potential analyzer (NanoBrook) manufactured by Brookhaven Instruments Corporation in the United States, a portable UV analyzer (WFH-204B) from Min Instruments, and a UPT-III-20 ultrapure water machine from Chengdu Super Pure Technology. The FA2004A electronic balance is from Shanghai Yueping Scientific Instrument Co., Ltd., and the toppette pipettor micropipette is produced by Zhengzhou Bokang Instruments and Equipment.

In addition,  $0.22 \mu\text{m}$  filter membranes, 100 mL, 250 mL, and 500 mL capacity bottles, various capacity beakers, 5 mL, 10 mL, 20 mL pipettes, culture dishes, and 500 Da dialysis bags and acid-resistant quartz colorimetric dishes were also used in the experiments.

### 2.2 Experimental Method

#### 2.2.1 Preparation of B-CDs

Weigh 1.5 g of chitosan using a balance and add it to 100 mL of deionized water. Mix thoroughly and then add 0.75 g of boric acid to the mixture. Transfer the mixture into a polytetrafluoroethylene-lined container, ultrasound for 5 minutes, and then place it in a high-temperature and high-pressure stainless steel reaction vessel. React at  $180^\circ\text{C}$  in a digital constant-temperature drying oven for 8 hours. After the reaction time, cool the solution, filter to obtain a carbon dot solution, and perform dialysis using a 500 Da dialysis bag for further purification for approximately 2 days, changing the water every 6 hours to remove molecular fragments and small-molecule reactants. Freeze-dry the solution to obtain brown solid carbon dot powder. Prepare a 2 g/L carbon dot solution. The synthetic route is shown in Figure 1.

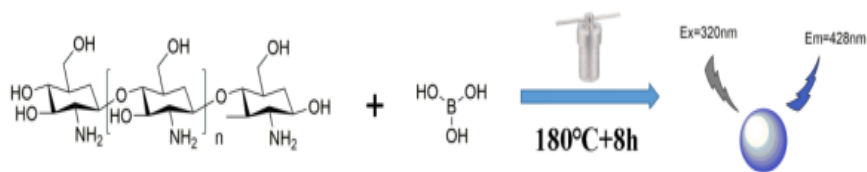


Figure 1: Schematic representation of the preparation of the B-CDs

### 2.2.2 Characterization of B-CDs

#### (1) Ultraviolet-Visible Absorption Spectra (UV-vis Spectra)

UV-vis absorption spectra were obtained by measuring a B-CDs solution with a concentration of 0.2 mg/mL using a UV-visible spectrophotometer. The scanning range was set from 200 to 800 nm.

#### (2) Fluorescence Spectra (FL Spectra)

The fluorescence spectra of B-CDs were measured using a fluorescence spectrophotometer with a scanning range of 300 to 800 nm.

#### (3) Fourier Transform Infrared Spectroscopy (FT-IR Spectra)

KBr crystals and dried B-CDs solid in an agate mortar were ground and pressed into pellets. The measurement range was from 500 to 4000  $\text{cm}^{-1}$ .

#### (4) X-ray Powder Diffraction Analysis (XRD)

The crystalline phase composition of the obtained samples was analyzed using a copper target as the X-ray source (tube voltage 40 kV, tube current 40 mA) in the X'Pert Powder equipment manufactured by Panalytical, within a scanning range of 5° to 90°.

#### (5) High-Resolution Transmission Electron Microscopy (HRTEM)

B-CDs were dissolved in water, sonicated for 30 minutes, and a small amount of the B-CDs solution was sprayed onto a copper grid. After natural drying, the appearance and morphology were observed.

## 3. Experimental Methods

### 3.1 Preparation of Carbon Dot Solution

Weigh 50 mg of carbon dot powder, dissolve it in deionized water, and make up to 100 mL in a volumetric flask, resulting in a 0.8 mg/mL carbon dot solution. During detection, use a pipette to transfer 500  $\mu\text{L}$  of the carbon dot solution and 1.5 mL of PBS buffer solution into a colorimetric dish.

### 3.2 pH Effect on Fluorescence Intensity

Prepare 1/15 mol/L solutions of potassium dihydrogen phosphate and sodium dihydrogen phosphate. Take a certain amount of each into a beaker, mix, and adjust the pH of the buffer solution using phosphoric acid or sodium hydroxide to adjust the pH from 3 to 12. During detection, transfer 500  $\mu\text{L}$  of the carbon dot solution and 1.5 mL of PBS buffer solution into a colorimetric dish.

### 3.3 Stability of Carbon Dots

Investigate the salt resistance of carbon dots by continuously adding NaCl (0-1 M) to the prepared carbon dot solution while detecting the fluorescence intensity of the system. Also, explore the antioxidant properties of carbon dots by continuously adding  $\text{H}_2\text{O}_2$  to the prepared carbon dot solution and measuring the fluorescence intensity change within 1 hour when the concentration reaches 500  $\mu\text{M}$ .

### 3.4 Photobleaching Resistance of Carbon Dots

Transfer 500  $\mu\text{L}$  of the carbon dot solution and 1.5 mL of PBS buffer solution into a colorimetric

dish, and then irradiate with 150 W UV light and 10 W xenon light for 1 hour and 2 hours, respectively.

#### 4. Determination of Fluorescence Quantum Yield

In this experiment, quinine sulfate with a known quantum yield of 54% was selected as the standard substance. It was dissolved in a 0.1 mol/L sulfuric acid solution ( $n=1.33$ ). The integrated area of the emission peak at the optimal wavelength and the ultraviolet absorbance of the prepared fluorescence carbon dot solution and quinine sulfate solution were measured. Based on the fluorescence spectra, the corresponding integrated fluorescence intensity was calculated. Finally, a fitting process was applied to the integrated fluorescence intensity and absorbance to determine the fluorescence quantum yield. The quantum yield of quinine sulfate under excitation at 320 nm is known to be 0.54. Therefore, the specific calculation formula for the quantum yield of the test solution is as follows:

$$\varphi = \varphi_x I A_x n^2 / I_x A n_x^2 \times 100\% \quad (1)$$

$\varphi$  represents the fluorescence quantum yield,  $I$  is the integrated area of the fluorescence spectrum,  $A$  is the absorbance value in the ultraviolet-visible range,  $n$  is the refractive index of the solution,  $x$  indicates the representative standard reference material [12].

#### 5. Conclusions

Water was collected from the natural springs behind the Liao Ning University campus, and a whitening cosmetic sample was purchased online. The extraction of  $Hg^{2+}$  from cosmetic samples was conducted following the procedures outlined in the national standard of the People's Republic of China (GB/T 7917.1-1987). The sample (2.00 g) was weighed and placed in a 100 mL colorimetric tube. Due to the presence of organic solvents such as ethanol in the sample, evaporation was performed in a water bath (avoiding drying). After adding 10 mL of nitric acid solution (density 1.42 g/mL) and hydrogen peroxide (30%), the mixture was left for 30 minutes and then heated in a boiling water bath for approximately 2 hours. After cooling to room temperature, it was diluted to 100 mL with 10% sulfuric acid (density 1.84 g/mL) and set aside. The  $Hg^{2+}$  content in the test solution was determined using ICP, and 200  $\mu$ L of the test solution was added to 1.3 mL of phosphate buffer solution (pH=4.0), followed by the addition of 500  $\mu$ L of the carbon dot solution into a colorimetric dish. The fluorescence intensity of the system was then measured. Standard curves were constructed using different concentrations of  $Hg^{2+}$  standard solution. Make the test curve of the sample solution and the standard curve. The pretreatment and extraction of the samples are carried out strictly according to the methods specified in the national standard.

#### References

- [1] Epa U. *Regulatory impact analysis of the clean air mercury rule*[J]. 2013-03-28]. [http://www.epa.gov/ttn/atw/utility/ria\\_final.pdf](http://www.epa.gov/ttn/atw/utility/ria_final.pdf), 2005.
- [2] Wang Y, Lou S, Liu X, et al. *Detection of trace metal ions in high-purity boric acid by online two-dimensional valve switching coupled with ion chromatography-inductively coupled plasma mass spectrometry* [J]. *Microchemical Journal*, 2020, 155: 104661.
- [3] Ding Q, Li C, Wang H, et al. *Electrochemical detection of heavy metal ions in water*[J]. *Chemical Communications*, 2021, 57(59): 7215-7231.
- [4] Fagadar-Cosma E, Lascu A, Shova S, et al. *X-ray structure elucidation of a Pt-Metalloporphyrin and its application for obtaining sensitive AuNPs-Plasmonic hybrids capable of detecting triiodide anions*[J]. *International Journal of Molecular Sciences*, 2019, 20(3): 710.
- [5] Wang C, Zhao Y, Lv K, et al. *Detection of mercury ions in aqueous solution by fluorescence spectrometry*[C]//IOP Conference Series: Materials Science and Engineering. IOP Publishing, 2020, 774(1): 012111.
- [6] Sun Z, Qu D. *The formation mechanism and fluorophores of carbon dots from the bottom-up route* [J]. *Materials Chemistry Frontiers*, 2020, 4(2): 400-420.
- [7] Carles G, Dabiri C, Mchirgui A, et al. *Uses of chitosan for treating different forms of serious obstetrics hemorrhages* [J]. *Journal of Gynecology Obstetrics and Human Reproduction*, 2017, 46(9): 693-695.
- [8] Divya K, Smitha V, Jisha M S. *Antifungal, antioxidant and cytotoxic activities of chitosan nanoparticles and its use as an edible coating on vegetables*[J]. *International journal of biological*

*macromolecules*, 2018, 114: 572-577.

[9] Zhan J, Peng R, Wei S, et al. Ethanol-precipitation-assisted highly efficient synthesis of nitrogen-doped carbon quantum dots from chitosan [J]. *ACS omega*, 2019, 4(27): 22574-22580.

[10] Yan X, Rahman S, Rostami M, et al. Carbon quantum dot-incorporated chitosan hydrogel for selective sensing of  $Hg^{2+}$  ions: Synthesis, characterization, and density functional theory calculation[J]. *ACS omega*, 2021, 6(36): 23504-23514.

[11] Song J, Zhao L, Wang Y, et al. Carbon quantum dots prepared with chitosan for synthesis of CQDs/AuNPs for iodine ions detection[J]. *Nanomaterials*, 2018, 8(12): 1043.

[12] Krysmann M J, Kelarakis A, Dallas P, et al. Formation mechanism of carbogenic nanoparticles with dual photoluminescence emission[J]. *Journal of the American Chemical Society*, 2012, 134(2): 747-750.