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N, B-doped carbon dots as a sensitive fluorescence probe for Hg^{2+} ions and 2,4,6-trinitrophenol detection for bioimaging

Qianghua Ye [a,1], Fanyong Yan [a,*,1], Dechao Shi [a], Tancheng Zheng [a], Yinyin Wang [b], Xuguang Zhou [a,*], Li Chen [a]

[a] State Key Laboratory of Hollow Fiber Membrane Materials and Processes, Key Lab of Fiber Modification & Functional Fiber of Tianjin, Tianjin Polytechnic University, Tianjin 300387, PR China
[b] TianJin Engineering Center for Safety Evaluation of Water Quality & Safeguards Technology, PR China

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ABSTRACT

Nitrogen and boron co-doped carbon dots (BCNDs1–3) were prepared from three kinds of borate via a facile hydrothermal method. The as-prepared BCNDs did not shift with the change of excitation wavelength and possess good water dispersibility, strong fluorescence emission with high fluorescent quantum yield of 29.01%, 51.42%, 68.28%, respectively. Subsequently, these BCNDs were exploited as excellent Hg^{2+} ion and 2,4,6-trinitrophenol (TNP) probe. The efficient selective detection of Hg^{2+} can be attributed to non-radiative electron/hole recombination annihilation through an effective electron transfer process and the detection of TNP can be attributed to the fluorescence resonance energy transfer process (FRET). The results show that the BCNDs2 is the most sensitive fluorescence probe for Hg^{2+} ions and TNP detection as low as Hg^{2+} 7.3 nM and TNP 0.35 μM compared with BCNDs1 and BCNDs3. The as-prepared BCNDs possess the advantages of good selectivity, fast response and a broad linear detection. They were applied to sensing and imaging of human umbilical vein endothelial cells, showing low cytotoxicity and good biocompatibility.

1. Introduction

Carbon dots (CDs) are a recently developed extraordinarily bright fluorescent carbon nanoparticles and exhibit several excellent advantages such as simple synthesis, high aqueous solubility, small size, tunable excitation and emission spectra, good biocompatibility, low toxicity, excellent photostability and environmental-friendly compared with traditional semiconductor quantum dots (QDs) [1–3]. CDs using in a broad range of promising applications have been demonstrated in bioimaging, medical diagnosis, catalysis, photovoltaic devices and sensor [4–13] especially for sensor application. For example, CDs have been used to detect cations, anions and organics including Ag^{+} [14], Cu^{2+} [15], Hg^{2+} [16], Fe^{3+} [17], I^{-} [18], PO_4^{3-} [19], dopamine [20], picric acid [21], hemin [22], 4-nitrophenol [23], dimethoate [24], melamine [25], bisphenol A (BPA) [26], tannic acid [27], L-cysteine [28], methotrexate [29] and amoxicillin [30].

The preparation approaches of CDs are generally classified into two categories "top-down" and "bottom-up" including laser ablation, electrochemical oxidization, chemical oxidation, hydrothermal carbonization and pyrolysis [31–34]. In order to improve fluorescence quantum yields of CDs, the surface passivation of CDs with polymers or small organic molecules has been used to gain strong photoluminescence. However, they need to use most of the organic reagents or catalyst and it is not environmentally friendly [35,36]. Heteroatom doping into CDs has been becoming a more powerful approach to improve the fluorescence properties of CDs such as nitrogen, aluminum, phosphorus, sulfur and boron [37–40].

Heteroatom doping with especially N-doping or co-doping with N and another element, has also been actively investigated to improve the fluorescence quantum yield of CDs [41,42]. The as-prepared N, S-CDs exhibited very high fluorescence quantum yields (73%) and excitation independent emission and the P-doped of PCQDs presented strong blue fluorescence with fluorescence quantum yield up to 25% [37,39]. Wang et al. [42] found that the luminescence brightness of the fluorescent carbon nanoparticles (CNPs) can be highly enhanced with the addition of $Al(NO_3)_3$, the results shown that the N element proved playing a key role in improving the photoluminescence. Shan et al. [43] found that the B-doped carbon quantum dots (BCQDs) can be used as a novel fluorescence sensing system for hydrogen peroxide and glucose detection. Among them, boron-doped CDs can obviously improve the fluorescence properties of CDs, the effects doping other elements such as boron of CDs is not very clear.

In this paper, we report the preparation of fluorescent N, B co-doped BCNDs1–3 from citric acid anhydrous (CA), ethylenediamine (EDA) by a facile, green, and low-cost hydrothermal carbonization method. The photoluminescent (PL) emission band of the as-prepared BCNDs1–3 did not shift with the change of excitation wavelength and exhibit high fluorescence quantum yield up to 29.01%, compared with other reported CDs [44]. Further study found that the prepared BCNDs1–3 can serve as an effective sensor for sensitive and selective determination

* Corresponding authors.
E-mail addresses: yfany@163.com (F. Yan), xaodayin@sohu.com (X. Zhou).
[1] These two authors contributed equally to this paper, and should share first authorship.

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of Hg^{2+} and TNP. As we all know, Hg^{2+} and TNP are harmful to our health. Mercury ions, especially water-soluble Hg^{2+}, can easily pass through skin, respiratory and gastrointestinal tissues, leading to DNA damages, mitosis impairment and permanent damages to the central nervous system [45,46]. TNP has been widely used as a military explosive, as a yellow dye and as an antiseptic [47]. It causes anemia, headache and liver injury [48]. Compared with the instrument test, BCNDs is a convenient, fast for the determination of Hg^{2+} or TNP probe.

2. Experimental Section

2.1. Chemicals and Apparatus

CA, EDA, boric acid, manganese borate, sodium tetraborate and ethanol purchased from the local company. Quinine sulfate (99%, suitable for fluorescence) was supplied by Aladdin. All other reagents were of analytical grade and used as received. The solutions of metal ions were prepared from $NaNO_3$, KNO_3, $AgNO_3$, $MgSO_4$, $CaCl_2$, $Mn(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}2H_2O$, $Ba(NO_3)_2$, $PdCl_2$, $Hg(NO_3)_2{\cdot}0.5H_2O$, $Pb(NO_3)_2$, $Fe(NO_3)_3{\cdot}9H_2O$, $Ce(NO_3)_3{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$. The solutions of Phenols were prepared from TNP, BPA, catechol, hydroquinone, *o*-nitrophenol, phenol, phloroglucinol, resorcinol and 1,3-dihydroxy-5-methylbenzene.

The FTIR spectra were recorded on a Fourier-transform infrared spectrometer (TENSOR37) and dry BCNDs1–3 powder was used as the sample with potassium bromide (KBr) as the matrix. The XPS spectra measurements were performed on an X-ray photoelectron spectroscopy (EDAX, GENESIS 60S). Transmission electron microscopy (TEM) measurements were performed on an electronic microscope (HITACHI, H-7650). The absorption spectra of the samples were measured on a UV–vis spectrometer (Purkinje, General TU-1901). Fluorescence spectra were obtained by an F-380 fluorescence spectrophotometer with the excitation wavelength of 350 nm. The fluorescence imaging was observed under an OlympusIX71 inverted fluorescence microscope with a 20× objective lens.

2.2. Preparation of N, B-doped Carbon Dots (BCNDs1–3)

BCNDs1–3 were synthesized through hydrothermal treatment of CA, EDA and three borates according to the recent reports [40,43,49]. In detail, CA, EDA and borate (borate represent sodium tetraborate, boric acid, manganese borate, respectively 1:3:1) were dissolved in deionized water (10 mL) and heated at 160 °C for 4 h in a poly(tetrafluoroethylene) autoclave (15 mL) respectively [50]. The autoclave was cooled down to room temperature, the solution was condensed by rotary evaporation and dissolved in ethanol. After removing the insoluble precipitate by centrifugation and the supernatant liquid was then loaded into dialysis bags (Mw = 3000) for dialysis against distilled water for 4 days. The final product was obtained by rotary evaporation and the obtained product was N, B co-doped CDs. These products were denoted as BCNDs (named BCNDs1, BCNDs2 and BCNDs3 from boric acid, manganese borate and sodium tetraborate, respectively).

2.3. Determination of Fluorescence Quantum Yield

QYs of the obtained BCNDs were determined by a relative method. Specially, quinine sulfate was selected as the reference. The QYs of a sample was then calculated according to the following equation [51]:

$$\varphi = \varphi_{st}(K/K_{st})(n/n_{st})^2 \quad (1)$$

where φ is the fluorescence quantum yield, K is the slope determined by the curves and η is the refractive index. The subscript "st" refers to quinine sulfate and n is the refractive index (1.33 for water and 1.36 for ethanol). To minimize reabsorption effects, absorption was always kept below 0.1 at the excitation wavelength.

2.4. Hg^{2+} Ion Detection

Based on the mechanism of fluorescence quenching, the as-prepared BCNDs were used to detect metal ions. Firstly, eighteen metal ions were applied in the process of experiment such as $AgNO_3$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$ and so on. In a typical detection experiment, 50 μL BCNDs solution was added into a 10 mL colorimetric tube, followed by a calculated amount of Hg^{2+} ions. The mixed solution was diluted to 10 mL with double distilled water. After reaction at room temperature for 15 min, fluorescence measurements were carried out with excitation and mission slit width of 5 and 5 nm, the excitation wavelength was 350 nm.

2.5. TNP Detection

A stock solution of phenols (1 mM) was prepared in double distilled water. Eight phenols were dissolved in double distilled water to afford 1×10^{-3} mol L^{-1} aqueous solution. All samples were prepared at room temperature. Fluorescence measurements were similar with Hg^{2+} ions detection.

2.6. Quenching Data Analysis

The quenching of fluorescence by Hg^{2+} or TNP was described using the Stern-Volmer equation:

$$F_0/F = 1 + K_{sv}C \quad (2)$$

where K_{SV} is the Stern-Volmer quenching constant, C the concentration of Hg^{2+} or TNP, F_0 and F the PL intensity of BCNDs without Hg^{2+}/TNP and with different concentration of Hg^{2+}/TNP respectively. The detection limit used the equation $3\sigma/m$, where σ is the relative standard deviation (n = 9) and m the slope of calibration curve as described in detail [52,53].

2.7. FRET Mechanism Analysis

The FRET mechanism of TNP detection was described using the Förster nonradiative energy transfer theory, Förster radius R_0 was expressed as follows Eq. (3) in angstroms (Å) [54]:

$$R_0 = 0.2108 \times \left[K^2 \times \Phi_D \times {n_R}^{-4} \times J(\lambda)\right]^{1/6} \quad (3)$$

where K^2 ($K^2 = 2/3$) is the factor describing the relative orientation of the donor to the acceptor molecule; n_R is the refractive index of the medium and Φ_D is the quantum yield of the donor. J(λ) is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor using Eq. (4):

$$J(\lambda) = \int_0^\infty F_D \times \varepsilon_A(\lambda) \times \lambda^4 \, d\lambda \quad (4)$$

$F_D(\lambda)$ is the normalized fluorescence intensity of the donor in the absence of acceptor; ε_A (λ) is molar extinction coefficient of the acceptor, and λ is wavelength.

2.8. Cellular Imaging

Human umbilical vein endothelial cells (HUVEC) were cultured in medium supplemented with 10% Fetal Bovine Serum in an atmosphere of 5% CO_2 at 37 °C. The cells were then incubated with the 2 mg mL^{-1} BCNDs in the culture medium for 0.5 h, and the medium was removed and the cells washed with phosphate buffer solutions (PBS) 3 times.