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Fluorescent nitrogen and sulfur co-doped carbon dots from casein and their applications for sensitive detection of Hg^{2+} and biothiols and cellular imaging

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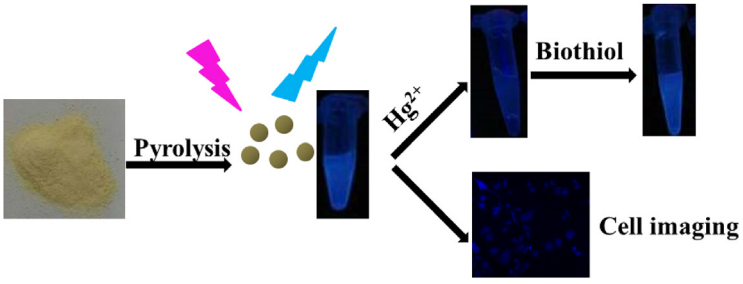
HIGHLIGHTS

- Casein was firstly used as the sources of carbon, nitrogen and sulfur for the synthesis of NSCDs using a one-step pyrolysis strategy.
- The NSCDs displayed a blue emission with quantum yield of 31.8%, good aqueous solubility, photostability and biocompatibility.
- The NSCDs were used as a probe for rapid and sensitive detection of Hg^{2+} with the limit of detection (LOD) of 6.5 nM.
- The NSCDs-Hg^{2+} system was employed as a fluorescent sensor for sensitive detection of biothiols.
- The NSCDs were used as effective fluorescent probes in cellular imaging without noticeable cytotoxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Fluorescent nitrogen and sulfur co-doped carbon dots (NSCDs) were synthesized by a one-step pyrolysis strategy using casein as carbon, nitrogen and sulfur sources, and characterized by UV–vis spectrum, fluorescent spectrum, X-ray photoelectron spectroscopy (XPS) and FT-IR, etc. The synthesized NSCDs displayed a blue emission under ultraviolet illumination with a quantum yield of 31.8%, and a good aqueous solubility, photostability and biocompatibility. It was found that the fluorescence intensity of NSCDs could be selectively quenched by Hg^{2+}, so NSCDs was used as an effective probe for the detection of Hg^{2+}. The linear range and the limit of detection (LOD) of the fluorescent sensor based on NSCDs for the detection of Hg^{2+} were 0.01–0.25 μM and 6.5 nM, respectively. Spiked water samples were detected by the sensor with the recovery of 95.4–106.3% and relative standard deviation (RSD) of 3.6–8.6%. It was also observed that the quenched NSCDs-Hg^{2+} system could be restored by the addition of biothiols such as L-cysteine (Lcy), homocysteine (Hcy) and glutathione (GSH), thus NSCDs-Hg^{2+} system was employed as a fluorescent sensor for the detection of biothiols. The linear range and LOD of the NSCDs-Hg^{2+} system were 1–10 μM and 23.6 nM for Lcy, 0.2–2.5 μM and 12.3 nM for Hcy, and 0.1–2.0 μM and 16.8 nM for

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Detection
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GSH, respectively. The NSCDs-Hg^{2+} system was applied for the detection of biothiols in serum samples with satisfied results. In addition, the study in vitro imaging HeLa cells revealed that the synthesized NSCDs could be used as effective fluorescent probes in cellular imaging without noticeable cytotoxicity.

1. Introduction

Over the past two decades, quantum dots (QDs) become one of the most extensively optical sensing nanomaterials in bioimaging [1] and the detection of nucleic acids [2], enzymes [3], proteins [4], metal ions [5] and other small molecules [6] due to their high emission quantum yields and size tunable emission profiles. However, serious toxicity of QDs even at relatively low concentrations [7] and their superior photophysical features usually observed in organic solvent restrict tremendously their analytical potential [8], so researchers pay their attentions on methods to make these luminescent QDs water-soluble and biocompatible, such as surface passivation with protective layers [9,10]. But these protocols are compromised by reducing their photoluminescence efficiency and the time-consuming, complicated, expensive processes.

Fluorescent carbon dots (FCDs), owing to their outstanding optical properties, low toxicity, good biocompatibility and robust chemical inertness [11,12], make them better than traditional quantum dots for many applications. Various methods have been demonstrated in preparation of FCDs, such as combustion [13], electrochemical oxidation [14], chemical oxidation [15], microwave heating [16] and so on. Among all of those preparation methods, thermal route is a promising and quite attractive one because it is particularly simple and efficient. In particular, to tune their electronic properties, surface and local chemistry, and to extend their applications, heteroatom (e.g., N, B, S, and Si) doping with or without surface modifications has also been actively investigated [17–20]. By using the precursors of L-cysteine and citric acid, N and S co-doped FCDs were prepared without using passivation reagents [21], or prepared from hair fiber [19], gentamycin sulfate [22] and rice [23]. Moreover, too much attention has been paid to their applications in bioimaging and sensing. Li et al. successfully prepared a novel high fluorescent nitrogen and sulfur co-doped carbon dots by one-step microwave-assisted method and were successfully applied to probe Hg^{2+} in living cells [24]. Despite these good examples, a better strategy to synthesize nitrogen and sulfur co-doped FCDs with high quantum yield and further developing their potential applications is still needed.

Hg^{2+} is one of the most dangerous and ubiquitous pollutants, and represents a serious threat to the environment and human health [25]. Therefore, there has been an increasing interest to develop highly efficient methods for the sensitive and selective detection of trace amounts of Hg^{2+}. Currently Hg^{2+} detection methods include atomic absorption/emission, spectrophotometry, inductively coupled plasma mass spectrometry, polarography and fluorescence assays [24,26–30]. Among them, fluorescence assays have been proven to be an alternative method for Hg^{2+} detection owing to their high sensitivity, good selectivity and fast analysis [31]. As one of fluorescence assays, CDs-based fluorescent nanoprobes with good water solubility has recently been actively explored for Hg^{2+} detection [24,32,33]. Although these CDs-based materials hold great promise in the detection of Hg^{2+}, much work is still needed to explore this system and extend its applications.

L-cysteine (Lcy), homocysteine (Hcy) and glutathione (GSH) are very common low-molecular-weight biothiols, playing crucial roles in biological processes such as reversible redox reactions and cellular functions [34]. Abnormal levels of them are linked with many ailments. Therefore, it is important to develop a sensitive and fast method for the determination of them. Recently, due to the features of high sensitivity, facile operation, real time and online detection, fluorescence detection has become more and more popular and much effort has been made to develop fluorescence sensors. The fluorescent sensors for the determination of biothiols based on CDs have also been developed in recent years [35–38].

Herein, we present a facile and general strategy to prepare nitrogen and sulfur co-doped CDs (NSCDs) by thermal treatment of casein at a relatively low temperature. It should be pointed out that the starting materials of NSCDs synthesis are non-toxic, environmentally friendly and biocompatibility. The as-prepared NSCDs exhibit blue emission under ultraviolet illumination with a high quantum yield, good aqueous solubility, photostability, biocompatibility and cellular labeling capability. Fluorescent intensity of the NSCDs is quenched by Hg^{2+} and the quenched NSCDs-Hg^{2+} system can be restored by the addition of biothiols, thus the fluorescent sensors using NSCDs and NSCDs-Hg^{2+} as the probes are used for the detection of Hg^{2+} and biothiols. Also, the prepared NSCDs are applied in bioimaging.

2. Experimental

2.1. Chemicals and materials

Casein, mercuric chloride ($HgCl_2$) and homocysteine (Hcy) were obtained from Sigma–Aldrich (Milwaukee, USA). Quinine sulfate, L-cysteine (Lcy), glutathione (GSH) and the other metal salts used here were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the reagents were used as obtained without further purification until and unless stated.

2.2. Apparatus

In all preparations, high-purity deionized water (18.2 MΩ) from a Dura series (The Lab Corporation, USA) was used. UV–vis absorption spectra were recorded with a spectrophotometer UV-2300 (Techcomp., Shanghai, China), and fluorescence spectra were taken on a spectrofluorometer F-2500 (Hitachi, Japan). The FTIR spectra were performed on a PerkinElmer Paragon 1000 FTIR spectrometer (Waltham, MA, USA). Transmission electron microscopy (TEM) was performed on a FEI Tecnai G-20 (FEI, Eindhoven, Netherlands), which was operated at an accelerating voltage of 200 kV. TEM samples were prepared by spraying a dispersion of NSCDs onto a Cu grid covered by a holey carbon film. Dynamic light scattering (DLS) and zeta-potential experiments were performed using a Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK). X-Ray photoelectron spectroscopy (XPS) measurements were carried out on a K-Alpha XPS spectrometer (ThermoFisher, E. Grinstead, UK), using Al Ka X-ray radiation (1486.6 eV) for excitation. For XRD analysis, the diffraction pattern was obtained using a PANalytical X'Pert^3 MRD diffractometer (Cu Kα radiation (λ = 1.54 Å) at 40 kV and 30 mA). The cellular fluorescence images were recorded by TCS SP5 II Confocal laser scanning microscope (Leica, Germany).