Journal of Photochemistry & Photobiology, B: Biology 186 (2018) 144–151

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Nitrogen and sulfur co-doped highly luminescent carbon dots for sensitive detection of Cd (II) ions and living cell imaging applications

Dan Gu, Liu Hong, Lei Zhang, Hao Liu, Shaoming Shang*

Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, PR China

ARTICLE INFO

Keywords:
Scallion
Carbon dots
Cd^{2+}
Cell imaging

ABSTRACT

In this work, we have developed a green, simple and fast one-pot microwave-assisted strategy for synthesis of nitrogen and sulfur co-doped fluorescent carbon dots (CDs) using scallion (SL) as the carbon source. Optical properties of the SL-CDs have been measured by UV-visible and fluorescent spectroscopy. The morphology of the prepared SL-CDs has been performed by transmission electron microscopy (TEM). Surface functionality and elemental composition of SL-CDs was analyzed by Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) spectra. The photoluminescent (PL) quantum yield of the obtained scallion carbon dots (SL-CDs) can reach as high as 18.6%. We further demonstrated that the SL-CDs can be used as fluorescent probes for detection of Cd^{2+} ions with a high sensitivity and an excellent selectivity. Linear relationships between the variation of the luminescent intensity of the SL-CDs before and after exposing the Cd^{2+} ions versus the concentration of Cd^{2+} ions in the range of 0.1–3.0 μM and 5.0–30.0 μM. The detection limit of Cd^{2+} ions can reach 15.0 nM. Moreover, the as-prepared SL-CDs exhibit negligible or extremely low cytotoxicity, which makes them be able to be used as fluorescent probes for living cell imaging. Overall, the prepared SL-CDs have promising applications in sensing of Cd^{2+} ions and in vivo or in vitro bioimaging.

1. Introduction

Pollution induced by various heavy metal ions has become a critical worldwide issue threatening the health of human beings and the ecosystem. Among miscellaneous heavy metal ions, Cd^{2+} has been proven to be a highly toxic heavy metal ion, which is widely applicated in weapons industry, metallurgy electroplating and agriculture [1–4]. Serious injury to the lung, kidney, bone, nervous system, and even certain cancers will be caused to those people continuously exposed to even a minute amount of Cd^{2+} ions through the englobement of polluted food or water [5–7]. Therefore, sensitive detection of Cd^{2+} is highly desired. Well-known detection approaches for Cd^{2+} ions include atomic absorption spectrometry [8], inductively coupled plasma mass spectrometry (ICP-MS) [9], spectrophotometric method [10], and stripping voltammetry. Although these methods have high sensitivity and multiplex detection capability, the complicated sample preparation procedures and the high cost and time-consuming detection process prohibits their applications in many real cases [11]. Therefore, developing simple methods that can selectively and sensitively detect Cd^{2+} ions is urgent. The fluorescent sensor has attracted extensive attention in recent years due to its merits including simplicity, cost effectiveness, high sensitivity, intuitiveness, and fast response. So far, diverse fluorescent probes have been developed relying on organic dye molecules, metal nanoparticles and semiconductor quantum dots (QDs). However, most of the above probes are usually either toxic or with low sensitivity and poor selectivity. Moreover, sensitive and highly selective detection of Cd^{2+} ions are troublesome since they have very similar chemical and physical properties to Zn^{2+} ions [12, 13]. Up to now, only a few highly selective fluorescence chemical sensor have been developed for Cd^{2+} detection [14–15]. So, it is still a challenge to explore new sensor for high selectivity and sensitivity towards Cd^{2+} against Zn^{2+} in aqueous solution.

After more than ten years' development, carbon dots (CDs) have grown to be an extraordinarily bright fluorescent probes with many unique advantages including good solubility in aqueous solutions, low toxicity, outstanding biocompatibility, and environmental friendliness, as well as good sensitivity and selectivity, when compared with organic dyes and traditional semiconductor quantum dots [16–19]. Moreover, carbon dots have been applied in a wide range of fields ranging from bioimaging [20–22] to catalysis [23] and sensor [24]. Previous studies have proved that not only surface functionalization/passivation, but heteroatom doping can improve the performance of CDs in the above-mentioned applications.

Nowadays, many efforts have been focused on controllable

* Corresponding author.
E-mail address: smshang@jiangnan.edu.cn (S. Shang).

https://doi.org/10.1016/j.jphotobiol.2018.07.012
Received 16 January 2018; Received in revised form 13 July 2018; Accepted 17 July 2018
Available online 18 July 2018
1011-1344/

synthesis of CDs doped with heteroatom, especially those doped with nitrogen and sulfur atoms with designable fluorescent properties [25–27]. Biomass has a plenty of carbohydrates that can supply a large amount of carbon atoms for the preparation of CDs. Meanwhile, the biomass usually contains different proteins and other molecules that can provide heteroatoms including N and S elements. Therefore, biomass has been used to prepare CDs such as bee pollens [28], hair [29], banana juice [30], winter melon [31] and potato [32].

Here, considering plentiful carbon, nitrogen, oxygen and sulfur elements existed in the proteins of scallion (SL), other than the vitamin, amino acid, carbohydrates, niacin, and lipid, we explored green fabrication of CDs doped with N and S atoms with controllable luminescent properties using scallion as a precursor. More importantly, the synthetic N and S co-doped SL-CDs were used to differentiate Cd^{2+} ions from other ions including Zn^{2+} with a high sensitivity. Moreover, these SL-CDs were employed in bioimaging applications benefited from their strong luminescence and biocompatibility. Overall, we developed a one-step microwave-assisted synthetic method to prepare N and S co-doped CDs with controllable and strong luminescence using scallion as a precursor. These SL-CDs can be used to detect and differentiate Cd^{2+} ions with a high sensitivity and also be adopted in bioimaging applications.

2. Experimental

2.1. Materials and Characterization

Scallions were bought from a local supermarket and washed several times with deionized water before usage. $HgCl_2$, $BaCl_2.2H_2O$, $CuCl_2$, $Pb(NO_3)_2$, $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CaCl_2$, $MgCl_2.6H_2O$, $Al_2(SO_4)_3.18H_2O$, $3CdSO_4.8H_2O$, CsCl, HCl, NaOH, and NaCl were purchased from Sinopharm Chemical Reagent Co.,Ltd., (Shanghai, China), which are used as received without further purification. The standard solutions of Cd^{2+} were purchased from National Center of Analysis and Testing for Nonferrous Metalsand Electronic Materials (Beijing, China). Deionized water was used in all experiments. Every chemical possessed the analytical quality and was utilized with no additional purification.

The morphology and structure of the SL-CDs were observed by a JEOL JEM-2100 high-resolution transmission electron microscope (Tokyo, Japan) with an accelerating voltage of 200 kV. The fluorescence spectra of the SL-CDs were recorded in a quartz cuvette (10 mm × 10 mm) on a Varian Cary Eclipse spectrofluorometer (Palo Alto, CA, USA) with excitation and emission slit width at 5 nm. UV–Visible absorption spectrum was measured by using a TU-1901 UV–Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., china). FTIR spectrum was performed on a FALA2000104 spectrophotometer. The fluorescence lifetime was measured using a Delta flex UltraFast lifetime spectrofluorometer (Horiba Jobinyvon IBH Inc., UK). X-ray photoelectron spectroscopy (XPS) was obtained using a Kratos AXIS Ultra DLD spectrometer (Shimadzu, Japan) equipped with a mono X-Ray source Al Kα excitation (1486.6 eV). The reaction was carried out in a G80F23CN2P-B5 (R0) microwave oven (Galanz Microwave Oven Co., Ltd., China) with power of 800 W. The size distribution of the as-prepared SL-CDs was revealed by dynamic light scattering (DLS) measurements using a Nano ZS90 instrument (Malvern).

2.2. Preparation of SL-CDs

In a typical synthesis, 10.0 g of freshly chopped scallions was introduced into a beaker and then transferred to a domestic 800 W microwave oven. Then the oven was heated for 4 min. Subsequently, 50 mL of deionized water was poured into the reaction mixture after it cooled to room temperature naturally. In consequence, the obtained turbid liquid was centrifuged at 8000 rpm for 10 min to remove those large particles. The resultant transparent supernatant was collected and filtered using a polytetrafluoroethylene (PTFE) syringe filter with 0.22 μm pore size on average. By performing the filtration step, a dark-brown solution was obtained. The dark-brown solution was dialyzed in deionized water though a dialysis membrane (i.e., 1000MWCO) for 3 days. The deionized water was changed for every 4 h. Eventually, high quality carbon dots were fabricated. Dry SL-CDs were obtained by lyophilizing the transparent and brown aqueous solution.

2.3. Quantum Yield Evaluation of the Photoluminescence from SL-CDs

The quantum yield (QY) of the photoluminescence from the SL-CDs was determined according to a previously reported procedure with quinine sulfate (whose QY is 54% [33] when dissolved in 0.1 M H_2SO_4) as a reference. To suppress the reabsorption effects, absorbance in the fluorescence cuvette of 10 mm thickness were maintained smaller than 0.1 at the excitation wavelength of 360 nm [34]. The following equation was used to evaluate the QY of the SL-CDs:

$$\varphi_x = \varphi_{re} \times (I_x/I_{re}) \times (A_{re}/A_x) \times (n_x/n_{re})^2.$$

where φ indicates the QY, I suggest the calculated integrated fluorescent emission intensity, A denotes the optical intensity at excitation wavelength, n implies the refractive index of the solvent. The subscript "x" refers to the sample and "re" refers to the reference with predetermined QY. In our case, $n_x = 1.33$ and $n_{re} = 1.33$.

2.4. Detection of Cd^{2+} Ions Using SL-CDs

Typically, 10 μL of SL-CDs dispersion was introduced into acetate buffer solutions (pH = 5). Then, Cd^{2+} ions were introduced into the above solution at different concentrations. The photoluminescent (PL) spectra of the solution contained Cd^{2+} ions were recorded after the solution stored for 3.0 mins at room temperature. The detection selectivity of SL-CDs in detecting Cd^{2+} ions was evaluated by measuring the PL spectra of the solution contained different control ions. The fluorescent intensities from the SL-CDs in the inexistence (F_0) or existence (F) of the control ions were measured. The selectivity and the sensitivity in sensing Cd^{2+} ions were measured for three times and the average values were presented for accuracy.

2.5. Cellular Toxicity Test and Cell Imaging Applications

The MTT assay method was used to study the cytotoxicity of the SL-CDs to A549 cells. Human lung adenocarcinoma A549 cells were first seeded into a plate with 96 wells at a concentration of about 2×10^3 cells per well and then cultured at 37 °C for 24 h under a 5% CO_2 atmosphere. In consequence, different amounts of SL-CDs were introduced into each well and incubated for another 24 h. Consequently, 10 μL of MTT solutions (at a concentration of about 5 mg/mL in a phosphate buffered saline solution) were added to each well and incubated for 4 h at 37 °C. The culture medium was removed before pipetting 100 μL of DMSO to each well followed by shaking for 10 mins. At last, a microplate reader was used to measure the optical density of the mixture at 570 nm wavelength.

In the propagation period, the A549 cells were dispersed in 24 replicate wells and incubated at 37 °C for 24 h in an incubator filled with 5% CO_2. The culture medium was replaced by a fresh medium containing SL-CDs at a concentration of 50 μg/mL. The A549 cells were incubated for another 2 h in the medium composed of SL-CDs. The A549 cells were washed three times with a PBS buffer solution before imaging with an inverted Olympus IX51 fluorescence microscope.