Journal of Luminescence 187 (2017) 274–280

Contents lists available at ScienceDirect

Journal of Luminescence

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

# A fluorescence probe based on the nitrogen-doped carbon dots prepared from orange juice for detecting $Hg^{2+}$ in water

Zhili Li [c], Ying Zhang [a,b], Qianqian Niu [a,b], Mingyao Mou [a,b], Yi Wu [a,b], Xiaoxuan Liu [a,b], Zhengyu Yan [a,b,*], Shenghua Liao [a,b,*]

[a] Department of Analytical Chemistry, China Pharmaceutical University, 24 Tongjia Lane, Gulou District, Nanjing 210009, People's Republic of China
[b] Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, 24 Tongjia Lane, Gulou, People's Republic of China
[c] Changzhou Vocational Institute of Engineering, Changzhou 213164, People's Republic of China

ARTICLE INFO

*Article history:*
Received 21 September 2016
Received in revised form
15 March 2017
Accepted 15 March 2017
Available online 18 March 2017

*Keywords:*
N-CDs
Orange juice
Ethylenediamine
Cytotoxicity
$Hg^{2+}$ detection

ABSTRACT

An excellent biocompatible nitrogen-doped carbon dots (N-CDs) was successfully synthesized from orange juice and ethylenediamine by hydrothermal decomposition method. The as-prepared N-CDs were mono-dispersed spherical nanoparticles with a narrow size distribution of 0.5–3.0 nm and showed a good dispersion and stability in aqueous solution with the pH value ranging from 3.0 to 13.0. Photoluminescence spectra of as-prepared N-CDs demonstrated that the fluorescence intensity of N-CDs was increased with the doped nitrogen atoms and the FL-QY (fluorescence quantum yield) of N-CDs was up to 31.7%. Compared with Gly-CQDs(CQDs synthesied by Gly), which were prepared from chemical carbon source *via* hydrothermal decomposition method, the as-prepared N-CDs showed much lower cytotoxicity for Human THP-1 macrophage cells. These results indicated N-CDs prepared by our proposed method have excellent compatibility and more suitable for the application in biolabeling and bioimage. Due to the fluorescence quenching of N-CDs by mercury (II) ion ($Hg^{2+}$), a sensitive and selective method was developed for detecting $Hg^{2+}$. The results indicated that the fluorescence intensity ratio of N-CDs was proportional to the concentration of $Hg^{2+}$ in the range from 4.0 μg/mL to 32.0 μg/mL and the recovery of spiked samples was ranged from 102.0% to 103.0%, which hinted our proposed method has a good sensitivity and accuracy and was suitable for detecting $Hg^{2+}$ with satisfactory in tap water.


## 1. Introduction

As novel carbon-based nanoparticles, carbon quantum dots (CDs) have attracted tremendous attention due to their unique photoelectronic properties, such as high quantum yield [1], low toxicity [2], good biocompatibility [3], high fluorescent stability [4], broaden and continuous excitation spectrum [5], high resistance to photobleaching [6], etc. Due to these advantages, CDs have been applied in various fields including analytical chemistry [7], bioimaging [8], pharmacy [9],etc.

Nowadays, a variety of methods were explored to prepare CDs. These methods can be classified into two categories: "top-down" and "bottom-up". In the former, the CDs were formed in the process of the discharge [10], laser ablation [11], electrochemical oxidation [12] of carbon-based nanomaterials. But, these methods have some drawbacks, such as unattainable or expensive raw materials and special instruments. Meanwhile, CDs were also prepared from the oxidation of carbon sources (such as petroleum coke, rose flowers, gelatin, etc) *via* chemical oxidation [13], microwave-assisted [14], solvothermal [15], and hydrothermal [16] in the latter. However, the fluorescence quantum yield of CDs synthesized by these methods is relatively lower. It is necessary that the surface of CDs was passivated for enhancing the fluorescence intensity by adding strong acid, which would bring secondary hazards for environment.

Recently, many papers reported that CDs can be synthesized using sustainable natural biomass as carbon sources instead of chemical carbon sources. These biomass carbon sources included soymilk [17], watermelon peel [18], egg [19], apple juice [20], jinhua bergamot [21], orange juice [22], and so on. In these reports, the carbon sources are easy to obtain in markets and the protocols for preparing CDs is cheap and green and sustainable. However, the FL-QY of prepared CDs is still needed be improved [23,18,19]. Thus, it is a challenge to develop a green and sustainable method for preparing CDs with good performance in photoelectronic properties and biocompatibility. Some research presented that the fluorescence properties of CDs were significantly

* Corresponding authors at: Department of Analytical Chemistry, China Pharmaceutical University, 24 Tongjia Lane, Gulou District, Nanjing 210009, China.
*E-mail addresses:* yanzhengyujiang@126.com (Z. Yan), liaoshenghuacpu@hotmail.com (S. Liao).

http://dx.doi.org/10.1016/j.jlumin.2017.03.023
0022-2313/

improved by doping N into the surface of CDs, in which the organic small molecules containing amino groups was used as co-precursors. Commonly, these co-precursors include ethylenediamine [5], amide sodium ($NaNH_2$) [17], urea [24] or branched polyethylenimine [25]. The reason is probable that the large conjugated carbon structure of CDs was broken and the surface defects of CDs was induced because of the doping of N atom [26]. To our knowledge, there is a few groups that report N-doped CDs prepared from natural biomass materials [22].

Herein, we proposed a simple and efficient method for synthesizing N-doped carbon quantum dots (N-CDs) by hydrothermal decomposition method, in which orange juice was used as carbon sources and ethylenediamine was used as nitrogen sources. The FL-QY of obtained N-CDs reached to 31.7%, which is higher than that of CDs [18,23] or N-CDs [27,28] prepared by others methods. To evaluate the biocompatibility of N-CDs, the cytotoxicity for Human THP-1 macrophage cells was investigated by 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazoliumbromide (MTT) assay. Besides, a sensitive and selective method for detecting $Hg^{2+}$ in water were developed because the fluorescence intensity of N-CDs was quenched by $Hg^{2+}$.

## 2. Materials and methods

### 2.1. Materials

All the fruit was purchased from the local market (Nanjing, China). Ethylenediamine was purchased from Sinopharm Chemical Reagent Co., Ltd. Silica gel was purchased from Qingdao Marine Chemical Co., Ltd. Citric acid was purchased from Nanjing Chemical Reagent Co., Ltd. Glycine was purchased from Zhuyanshengwu Co., Ltd. THP-1 cells line was obtained from cell bank of Chinese Academy of Sciences. RPMI1640 culture medium and fetal bovine serum (FBS) was purchased from Hyclone Company (USA). The inducer of phorbol ester (PMA P1585) was purchased from Sigma company (USA). Other reagents were analytic grade and used without further purification.

### 2.2. Instruments

The transmission electron microscope (TEM) image of N-CDs was obtained with JEOL JEM-2100 transmission electron microscope at 220 kV. The PL and absorption spectra were recorded with RF-5301 spectrophotometer (Shimadzu, Japan) and UV-2100 UV/Vis spectrophotometer (Shimadzu, Japan), respectively. The Fourier transform infrared spectra (FT-IR) of N-CDs was recorded with a FTIR-8400S spectrometer (Shimadzu, Japan) ranging from 500 $cm^{-1}$ to 4000 $cm^{-1}$. X-ray Photoelectron Spectroscopy (XPS) was investigated by using Thermo ESCALAB 250XI photoelectron spectrometer with a mono X-ray source Al Kα excitation (1486.6 eV). The cytotoxicity of N-CDs and Gly-CQDs was recorded by Mode 1680 microplate reader (Bio-Rad, Hercules, USA). The confocal fluorescence images of N-CDs and Gly-CQDs labeled cells were acquired with IX71inverted fluorescence microscope (Olympus, Japan) under ambient conditions.

### 2.3. Synthesis of N-CDs

The fluorescent N-CDs were synthesized via one-step hydrothermal decomposition method using orange juice as carbon source and ethylenediamine as nitrogen source respectively. Typically, the fruit was cut into small pieces and was put into a juice extractor to obtain orange juice. Then, 10 mL of orange juice and 5 mL of ethylenediamine (1 mol/L) in aqueous solution were transferred into a 20 mL Teflon-autoclave, the mixture solution was heated to 200 °C and kept for 11 h. The product was cooled to room temperature and filtered by medium speed filter paper. The filtrate was washed by adding equal volume of dichloromethane to remove liposoluble substance. The operation was repeated several times and the obtained supernatant was the crude solution containing N-CDs.

### 2.4. Purification of N-CDs

The N-CDs were purified by column chromatography, in which the stationary phase is silica. Firstly, the crude solution above mentioned was dried to remove water by rotary evaporator and the obtained sample powders were loaded into the column chromatography. Then the column was eluted to remove less fluorescent impurities with the mixed solution of dichloromethane and methanol (2:1, V/V) until the color of eluted solution was transparent colorless. Finally, the column was eluted with purified water and the eluted solution was collected. The collected solution was centrifuged at 13,000 rpm for 15 min to remove silica. The obtained supernatant was evaporated and the CDs powders were obtained for further use.

### 2.5. Synthesis of Gly-CQDs

Following our group reported method [29], the Gly-CQDs were synthesized using citric acid and glycine as precursors. Briefly, the citric acid and glycine were dissolved into water and then transferred into Teflon-lined autoclave and were heated at 200 °C for 4 h. The as-synthesized Gly-CQDs were purified and used for the comparison with N-CDs in the following cell cytotoxicity study.

### 2.6. The cytotoxicity study of N-CDs and Gly-CQDs

MTT test was experimented according to the methods reported in literature [30,31] and the THP-1 macrophage cells were selected as model cells to value the biocompatibility of N-CDs. In detailed, the THP-1 cell lines were cultivated in RPMI-1640 media supplemented with 10% heat-inactivated fetal bovine serum. THP-1 were plated and stimulated with 50 ng/mL of phorbol-12-myristate-13-acetate (PMA) for 48 h to induce differentiation into macrophages. Then, two kinds of CDs with varying of concentration (0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.5, 25.0 mg $mL^{-1}$), which were sterilized by filtration membrane in advance, were added and each concentration was set to 3 complex holes. After being incubated with THP-1 for 24 h, these cells were washed by sterile PBS three times to remove the excess of CDs. The cells were added with MTT solution and incubated 4 h in dark condition. Then the 96-well culture-treated plate were placed in Mode 1680 microplate reader (Bio-Rad, Hercules, USA) and oscillated for 10 min. The absorbance at 490 nm were measured and the viability of THP-1 cell were calculated.

### 2.7. Fluorescence quenching of N-CDs by $Hg^{2+}$

First, 0.5 mL of CDs solution (60.0 μg/mL) and 1.0 ml of PBS buffer solution (pH=10.0) were added into 10 mL volumetric flask. Then, varying volume of $Hg^{2+}$ (0.01 mol $L^{-1}$) stocked solution was added and the mixed solution was diluted to the mark respectively. After being reacted for 6 min, the corresponding fluorescence spectra of mixed solution was recorded at excitation wavelength of 360 nm by fluorescence spectrophotometer at ambient conditions.