One-step fabrication of nitrogen-doped fluorescent nanoparticles from non-conjugated natural products and their temperature-sensing and bioimaging applications

Xiaoling Zeng [a,b], Xinmei Yang [a,b], Feiming Li [a,b], Jie Ma [a,b], Yanping Lin [a,b], Bixia Yao [a,b], Lizhang Huang [c], Wen Weng [a,b,*]

[a] College of Chemistry and Environment, Minnan Normal University, 36 Xianqian Straight Street, Xiangcheng District, Zhangzhou 363000, China
[b] Key Laboratory for Analytical Science of Fujian Province University, Zhangzhou 363000, China
[c] Zhangzhou Product Quality Supervision Institute, Zhangzhou 363000, China

ARTICLE INFO

ABSTRACT

A facile solvothermal method was used to prepare N-doped fluorescent nanoparticles (NFNPs) at gram scale from tartaric acid/citric acid/ethylenediamine using oleic acid as the reaction medium. The quantum yield of the obtained fluorescent nanoparticles could reach 48.7%. The NFNPs were characterized by multiple analytical techniques. By combining with the circular dichroism (CD) spectra, the structure and the origin of photoluminescence of the NFNPs were discussed. The fluorescent intensity of the obtained NFNPs had remarkable stability, and exhibited a reversible temperature-dependent enhancement/quenching. The products with low cytotoxicity could be introduced into the target cells for in vitro bioimaging.

1. Introduction

Fluorescent nanoparticles (FNPs) have received much attention in recent years because of their promising applications in many fields such as bioimaging, biosensing, optoelectronic devices and photocatalysis. As the toxicity of the semiconductor quantum dots (QD) containing cadmium or other heavy metals has been widely realized, it is significant to search for nontoxic alternative QD-like fluorescent nanomaterials [10,11,21,22,31,38]. Carbon-based nanoparticles, which include carbon dots, nanodiamonds, carbon nanotubes and fluorescent graphene, are regarded as appropriate candidates [1–5,16,17,20,24,30]. Owing to exceptional advantages of chemical stability, biocompatibility, high water-solubility, sufficient fluorescence quantum yield (QY) and low toxicity, carbon dots have drawn most attention. To date, a variety of techniques have been developed to prepare carbon dots. The established techniques can be classified into two main types: top-down strategy and bottom-up strategy. Top-down strategy includes arc discharge, laser ablation, strong acid oxidation and electrochemical oxidation. Bottom-up strategy includes hydrothermal treatment,

ultrasonic method, microwave method and supported method [19,25,32,33,35,40].

A unique property of carbon dots is that the bare carbon dots are not luminescent [15]. When passivated with polymer chains or doped with heteroatoms, the quantum yield of the carbon nanoparticles could be greatly improved [7–9,13,18,27,28,37,39]. Heteroatom-doped carbogenic nanoparticles have attracted much attention of people very recently because of their high quantum yield and desirable diversity. Though some significant progress has been achieved, developing simple methods suitable for synthesizing heteroatom-doped fluorescent nanoparticles is still very meaningful [26,36].

On the other hand, fabrication of safer and efficient fluorescent nanoparticles from non-conjugated natural products is a meaningful strategy [4,5]. In this report, we prepared a kind of N-doped fluorescent nanoparticles (NFNPs) from a common chiral source, tartaric acid, coupled with citric acid by a simple one-pot solvothermal method at moderate temperatures. Tartaric acid and citric acid are small, "safe", and readily available non-conjugated molecules, and their multiple carboxyl and hydroxyl groups give them good dehydration trend at high temperature. The quantum yield of the obtained NFNPs could reach 48.7%, which was comparable to those of reported N-doped nanoparticles [22,23,28,35,38]. As the product could precipitate from the reaction media directly,

* Corresponding author at: College of Chemistry and Environment, Minnan Normal University, 36 Xianqian Straight Street, Xiangcheng District, Zhangzhou 363000, China.

the fabrication was easy to enlarge to gram scale. The obtained NFNPs exhibited low cytotoxicity to the target cells NIH3T3 within limits, and could be introduced into the cells for *in vitro* bioimaging. In recent years, different nanoparticle modalities such as fluorescence quantum dots, gold (Au), silica, carbon dots, etc., have been developed to visualize and monitor cellular and subcellular activities [14], we think that this research may have some contribution to the application in cell imaging.

2. Experimental section

2.1. Chemicals

L- and *D*-tartaric acid (TA), citric acid (CA) and ethylenediamine (EDA) were purchased from Alfa Aesar China (Tianjin). Oleic acid, *n*-hexane, ethanol and chloroform were analytical reagents. Ultrapure water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Preparation of the N-doped fluorescent nanoparticles (NFNPs)

A typical synthesis process is as follows. *L*-Tartaric acid (1.5 g), citric acid (1.5 g), ethylenediamine (5.0 mL) and oleic acid (30 mL) were placed in a three neck flask, and the mixture was heated at 220 °C for 30 min under vigorous magnetic stirring. Tartaric acid and citric acid were melted at about 170 °C, and the mixture turned from a colourless solution to a clear dark yellow solution. Certain brown solid emerged a few minutes after the reaction temperature reached 220 °C. After cooling, the solid was filtered and washed sufficiently with hexane, ethanol and chloroform. The product was dried at 50 °C by a vacuum desiccator. *L*-TA-CA-NFNPs (1.23 g) were obtained.

2.3. Characterization

Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were recorded using FEI Tecnai G2 F20 instruments (FEI). Elemental analysis was performed using a vario EL elemental analyzer (Elementar Analysensysteme GmbH). FT–IR spectra were recorded using a Magna-IR 750 Fourier transform infrared spectrometer (Nicolet). A UV-2550 spectrophotometer (Shimadzu) was used to record the UV–vis spectra of the FNPs in ultrapure water. The XPS spectra were recorded using a Thermo ESCALAB 250Xi multifunctional imaging electron spectrometer (Thermo Fisher). The photoluminescence spectra of the as-prepared FNPs were recorded using a Cary Eclipse PL spectrophotometer (Varian). The circular dichroism spectra were recorded using a J-815 CD spectrophotometer Spectra Manager (JASCO).

2.4. Quantum yield measurement

The quantum yield (Φ) of the obtained FNPs was measured by comparing the integrated photoluminescence intensities and the absorbency values of the FNPs with the reference quinine sulfate (QS). The quinine sulfate (literature $\Phi_R = 0.54$) was dissolved in 0.1 M H_2SO_4 (refractive index η_R of 1.33) and the FNPs was dissolved in ultrapure water ($\eta = 1.33$).

$$\Phi = \Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2}$$

where Φ is the quantum yield, I is the measured integrated emission intensity, η is the refractive index of the solvents, and A is the optical density. The subscript R refers to the reference. In order to minimize the re-absorption effects, absorbencies in the 1 cm cuvette should never exceed 0.1. The as-prepared products were all dissolved in ultrapure water. The characterizations were performed at room temperature unless otherwise noted.

3. Results and discussion

3.1. NFNPs derived from tartaric acid and ethylenediamine

The FNPs derived from tartaric acid showed low QY values (4.02% and 3.64% for *L*-TA-FNPs and *D*-TA-FNPs, respectively). The TA-FNPs had a stronger absorption than the starting materials in the UV region, and exhibited an apparent absorption in the visible region (Fig. S1A). The maxima emission peak was observed at 435 nm, at an excitation wavelength of 345 nm (Fig. S1B). It is worth of noting that the starting material, tartaric acid, shows very faint luminescence intensity at the same concentration of the FNPs.

Table S1 shows the influence of different proportions of tartaric acid and ethylenediamine on the QY value. The results showed that a small quantity of ethylenediamine (0.3 mL) did not cause the improvement of the QY value, though the nitrogen content reached 4.8% (Table S1, Serial 1). When the content of ethylenediamine was in the range of 0.6–2.5 mL, the obtained NFNPs were water-insoluble. Element analysis showed that these NFNPs contained about 45% of the carbon and 15% of the nitrogen (Table S1, Serials 2 and 3). The obtained NFNPs became water-soluble with the further increase of the content of ethylenediamine (Table S1, Serials 4–6). Too much amount of ethylenediamine would lead to low product yield. Compared to the water-insoluble NFNPs, the water-soluble NFNPs contained a little less amount of carbon and a higher amount of oxygen. The amount of nitrogen was almost identical for the water-soluble and water-insoluble NFNPs. The products derived from *L*- or *D*-tartaric acid had a similar element composition and quantum yield (Table S1, Serials 5 and 6). The highest QY value (15.6%) was achieved using 3.0 g *D*-tartaric acid and 5.0 mL ethylenediamine as the precursors (Table S1, Serial 6).

Fig. 1A shows the UV–vis absorption spectrum and excitation-dependent emission spectra of *L*-TA-NFNPs obtained from 3.0 g *L*-tartaric acid and 5.0 mL ethylenediamine. Compared with *L*-TA-FNPs, the UV–vis absorption spectrum of *L*-TA-NFNPs had a distinct peak centered about 322 nm, which can be attributed to the trapping of excited-state energy by the surface states results in strong emission [9]. The position of emission peak of the *L*-TA-NFNPs remained unchanged nearly when the excitation wavelength was in the range of 330–370 nm, and underwent a redshift gradually from 390 to 510 nm. The maxima emission peak was observed at 460 nm, at an excitation wavelength of 370 nm.

The TEM image (Fig. 1B) demonstrated that the obtained TA-NFNPs were monodispersed and spherical, the diameter of most NFNPs was in the range of 6–12 nm with an average diameter of 9.79 nm. The high-resolution TEM (HRTEM) images of the *L*-TA-NFNPs did not show any discernible lattice fringes, indicating the amorphous nature of the obtained NFNPs.

The surface element composition for the as-prepared *L*-TA-NFNPs was characterized by XPS further. The XPS spectrum shows three peaks at 284, 399 and 531 eV (Fig. S2A), which are attributed to C_{1s}, N_{1s} and O_{1s}. The atomic ratio of C_{1s}, N_{1s} and O_{1s} was 63.21%, 14.13% and 22.66%. The deconvolution of the C_{1s} spectrum of *L*-TA-NFNPs indicated the presence of three types of carbon bonds: sp^2 C=C or sp^3 C–C (284.78 eV), C–O or C–N (286.94 eV), and C=O or C=N (289.20 eV) (Fig. S2B). The deconvolution of the N_{1s} spectrum indicated the presence of two types of nitrogen bonds: C–N (398.40 eV) and N–H or C=N (400.07 eV) (Fig. S2C).

The ^{13}C NMR spectrum of the *L*-TA-NFNPs displayed three groups of peaks: 36–40, 72–75, and 160–180 ppm (Fig. S3), which could be assigned to methylene carbons stemmed from