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Sulfur and nitrogen binary doped carbon dots derived from ammonium thiocyanate for selective probing doxycycline in living cells and multicolor cell imaging

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ABSTRACT

A novel sulfur and nitrogen binary doped carbon dots (S,N-CDs) was synthesized by one-step manner through the hydrothermal treatment of citric acid (CA) and ammonium thiocyanate, and the procedures for biomedical applications, including probing doxycycline in living cells and multicolor cell imaging were developed. The obtained S,N-CDs are stable in aqueous solution, possess a very high quantum yield (QY, 74.15%) and good photostability. The fluorescence of S,N-CDs can be specifically quenched by doxycycline, providing a convenient turn-off assay of doxycycline. This assay shows a wide linear detection range from 0.08 to 60 μM with a low detection limit of 20 nM. The present method also displays a good selectivity. More importantly, the S,N-CDs have an excellent biocompatibility and low cytotoxicity, allowing the multicolor cell imaging and doxycycline detection in living cells. Consequently, the developed doxycycline methods is facile, low-cost, biocompatible, sensitive and selective, which may hold the potential applications in the fields of food safety and environmental monitoring, as well as cancer therapy and related mechanism research.

1. Introduction

Doxycycline, a tetracycline derivative with a wide range of antibacterial activities, is frequently used to treat many infections such as chronic prostatitis, respiratory tract infections, sinusitis [1] and sexually transmitted diseases [2]. Additionally, it also applied in the fields of veterinary medicine and animal nutrition. But the abuse of doxycycline may lead to an unsafe residue level in the environment or food samples. To protect the safety of consumers, the European Union, America, Canada and China have legally set the maximum residue level for various antibiotics. Consequently, developing efficiently methods for the detection of doxycycline concentration is of great importance in pharmaceutical preparations, food safety and environmental monitoring. In the past 20 years, numerous analytical techniques including chromatography [3-5], capillary electrophoresis [6], spectrophotometry [7,8], fluorescence [9], electrochemistry [10,11], coupled technique [12-16] were used for doxycycline monitoring. However, these methods commonly suffered from low sensitivity and/or complicated preparation. In addition to the anti-microbial activity, doxycycline also shows an anti-tumour property in the treatment of prostate, pancreatic and colon cancer [17-19]. Thus, detecting doxycycline in cancer cells may provide more useful and direct information for cancer therapy and related mechanism research. But the reports about doxycycline monitoring in living cells are still rare at present.

As fascinating carbon nanomaterials, fluorescent carbon dots (CDs) have attracted lots of research interests due to their excellent photostability, favorable biocompatibility, low toxicity without heavy metal ions and toxic elements, tunable fluorescence emission and excitation, and large Stokes shifts [18]. Based on these outstanding properties, CDs have been widely applied in the fields of bioimaging, biosensor and drug delivery [20-28]. Additionally, at present, great efforts were made to develop heteroatom-doped CDs for improving the optical and electrical properties of CDs. Boron, nitrogen, fluorine and sulfur were the commonly introduced doping chemical elements [29,30]. Among these heteroatom-doped CDs, sulfur and nitrogen co-doped CDs

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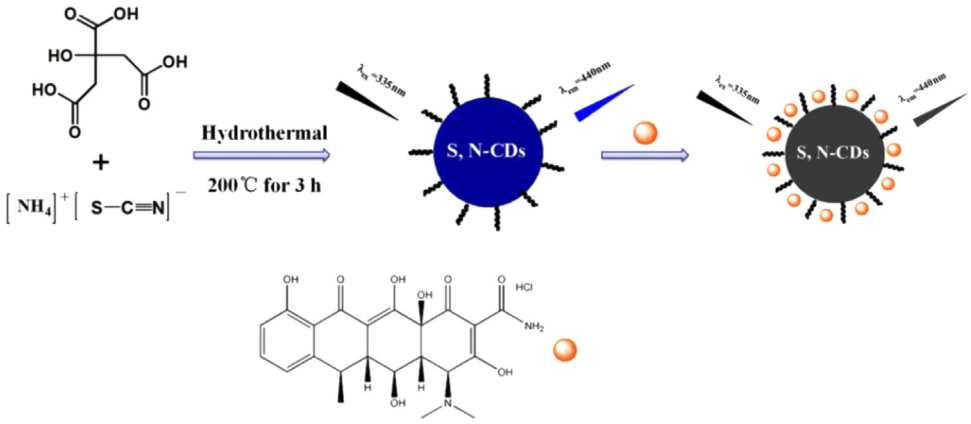

Scheme 1. Schematic illustration of S,N-CDs preparation and doxycycline detection.

were extremely attractive, which exhibited a high QY [31-33].

In this work, a novel low-cost strategy is developed for the synthesis of S,N-CDs by using CA and ammonium thiocyanate as precursors. The CA serves as the carbon source, while the ammonium thiocyanate provides sulfur and nitrogen. The as-obtained S,N-CDs are stable in aqueous solution and exhibit a very high QY (74.15%). The fluorescence can be specifically quenched by doxycycline. Thus, using S,N-CDs as fluorescent probes, a simple doxycycline detection was achieved (Scheme 1). The high QY of obtained S,N-CDs offers a high sensitive for doxycycline assay. There are few works about the CDs-based detection of tetracyclines [34,35], but each tetracycline derivative could induce a similar response signal in these works. The proposed S,N-CDs probe here exhibits an approving selectivity and can distinguish between doxycycline and tetracycline. The high sensitivity and selectivity may ensure a more accurate analysis of doxycycline in complex samples. Remarkably, the synthesized S,N-CDs possess high photostability and excellent biocompatibility, good stability under the entire physiological pH range and high ionic strength. Coupled with these outstanding features, S,N-CDs are promising in the cell imaging and doxycycline monitoring in living cells.

2. Experimental

2.1. Reagents and materials

CA, ammonium thiocyanate, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical materials were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). All reagents were of analytical grade and were used without further purification. Water was purified with a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA) and used throughout the work.

2.2. Synthesis of S,N-CDs

The S,N-CDs were prepared by thermal treatment of molecular organic salts with the mixed carbon source and the surface modifier in the single precursor. Briefly, 2.1080 g CA and 1.5439 g of ammonium thiocyanate were dissolved into 5 mL water. The solutions were heated hydrothermally in a Teflon-equipped stainless-steel autoclave at 200 °C for 3 h with a heating rate of 10 °C min^{-1}. The obtained S, N-CDs solution was adjusted to pH 7 with 1 M NaOH solution and centrifuged at 10,000 rpm for 20 min. A dialysis membrane (MWCO: 1 kDa; pore size: ca. 1.0 nm) was then used to separate the S,N-CDs from any residual unreacted species and obtained a brown solution. The solvent was removed with the aid of a rotary evaporator. Then the obtained S, N-CDs was dispersed into deionized (DI) water and preserved at a 4 °C for further characterization and use.

2.3. Instruments and characterizations

The morphology and microstructure of S,N-CDs were examined by high-resolution transmission electron microscopy (HRTEM) on a Tecnai G2 F20 microscope (FEI, Philips, Netherlands) with an accelerating voltage of 200 kV. The sample for HRTEM was made by dropping an aqueous solution onto a 300-mesh copper grid coated with a lacy carbon film. The X-ray diffraction (XRD) measurement was performed with a D/max-2500V/PC powder X-ray diffractometer (Rigaku, Tokyo, Japan). The X-ray photoelectron spectroscopy (XPS) spectra of the sample were measured on a ESCALAB 250Xi X-ray Photoelectron Spectroscopy (Thermo Scientific, Waltham, MA, USA). Fourier transform infrared spectroscopy (FT-IR) study was conducted from KBr pellets on a PerkinElmer FT-IR spectrophotometer (Perkin-Elmer, Norwalk, Connecticut, USA). Elemental analyses (C, H, N, S) were carried out on a Perkin Elmer Series II CHNS/O 2400 elemental analyzer (Perkin-Elmer, Norwalk, Connecticut, USA). Fluorescence lifetime experiments were performed by a FL3-P-TCSPC time-resolved fluorescence spectrometer (Horiba Jobin Yvon, Longjumeau, France). Raman spectra were obtained on a Raman Microscope (Renishaw, Gloucestershire, UK). All fluorescence spectra were obtained on a LS-55 fluorescence spectrometer (Perkin-Elmer, Norwalk, Connecticut, USA). The emission spectra were recorded in the wavelength range of 400–600 nm. Ultraviolet–visible (UV–vis) absorption spectra were characterized by a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Cell imaging was examined by Zeiss LSM 710 confocal microscopy (Carl Zeiss, Oberkochen, Germany).

2.4. Measurement of fluorescence QY

Quinine sulfate (0.1 M H_2SO_4 as solvent; QY=0.54) was chosen as a standard. The QY of S,N-CDs (in water) were calculated according to the following equation:

$$\varphi_x = \varphi_{st} \times (I_x/I_{st}) \times \eta_x^2/\eta_{st}^2 \times (A_{st}/A_x)$$

where φ is the QY, I is the measured integrated emission intensity, η is the refractive index of the solvent, and A is the optical density. The subscript "st" refers to standard with known QY and "x" refers to the unknown samples. For these aqueous solutions, $\eta_x/\eta_{st}=1$.