Supporting Information

A novel N-doped carbon dots derived from citric acid and urea: fluorescent sensing for determination of metronidazole and cytotoxicity studies

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Reagents and materials

Citric acid, urea, metronidazole, L-Serine (L-Ser), L-Histidine (L-His), L-Threonine (L-Thr), and L-Phenylalanine (L-Phe) were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Quinoline sulfate, H_2SO_4, KCl, $MgCl_2$, $CaCl_2$, NaCl, and $NiSO_4$ were purchased from Beijing Chemical Plant (Beijing, China). HaCaT (Immortalized human epidermal keratinocyte cell line) was obtained from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Beijing, China).

Fetal bovine sera (FBS) were purchased from Zhejiang Tianhang Biotechnology Co., Ltd (Hangzhou, China) DMEM (Dulbecco's Modified Eagle Medium) with high glucose was acquired from Sigma-Aldrich (Missouri, USA) Trypsin-EDTA solution was purchased from Beijing Solabao Technology Co., Ltd. (Beijing, China).CCK-8 kitand PBS (phosphate buffered saline) were obtained from Hefei Lanjieke Technology Co., Ltd. (Anhui China). Cell culture flasks were purchased from Corning Life Sciences (North Carolina, USA). All chemicals were analytical pure and used without any further purification. The experimental water was all ultrapure water obtained from the ultrapure water preparation system.

Characterizations

The morphology of the N-CDs was investigated by the high-resolution Talos F200X G2 transmission electron microscope (ThermoFisher Scientific, Massachusetts, USA). X-ray diffraction (XRD) pattern of the N-CDs was recorded by the Bruker D8 FOCUS (Bruker, Massachusetts, USA). The element composition was analyzed by the ESCALAB250Xi X-ray photoelectron spectrometer (ThermoFisher Scientific, Massachusetts, USA). Functional groups on the surface of N-CDs were characterized by the NICOLET 380 FT-IR spectrometer (ThermoFisher Scientific, Massachusetts, USA). Ultraviolet-visible (UV-vis) absorption spectra of N-CDs were performed by the TU-1901 UV-visible spectrophotometer (Purkinje, Beijing, China) Fluorescence spectra were recorded by the RF-5301PC fluorescence spectrophotometer (Shimadzu, Kyoto, Japan). The photostability experiments of N-CDs were performed by the WD-9403E UV analyzer (Liuyi, Beijing, China) with the

power of 6W. The CO_2 incubator (Thermo Fisher Scientific, Massachusetts, USA) were used to grow HaCaT cell cultures. Cell cytotoxicity were measured by the AMR-100 microplate reader (Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China). LabRam HR Evolution (Horiba, Japan)

Synthesis of N-CDs

N-CDs were prepared by one-step hydrothermal synthesis method. Briefly, 0.5041g of Citric acid and 2.5140g of urea were mixed and dissolved in 15mL ultrapure water, then the mixture was transferred into a Teflon-lined autoclave and heated at 190℃ for 6h. Subsequently, the reaction solution was cooled down naturally to room temperature and centrifuged to remove large particles. Next, the solution was filtered through a 0.22 μm filter membrane and for 48 hours using a dialysis tubing (MWCO=500Da). Solid N-CDs were obtained by drying the dialyzed solution with the vacuum freezer. The obtained N-CDs was stored at 4℃ for further experiments.

Quantum Yield of N-CDs

To measure the quantum yield of N-CDs, quinine sulfate were used as the standard substance and the fluorescence quantum yield of N-CDs was calculated according to the following equation:

$$\Phi_x = \Phi_R \times \frac{I_x}{I_R} \times \frac{A_R}{A_x} \times \left(\frac{\eta_x}{\eta_R}\right)^2$$

Where subscripts R and x stand for reference and sample, respectively. Φ, η, I, and A refer to quantum yield, solvent refractive index, integrated emission intensity, and absorbance, respectively.

Detection of metronidazole

10 μL N-CDs solution was mixed with 1000 μL phosphate buffer (pH7.4), different volumes of 10.00 μM metronidazole solutions was then added to the N-CDs solution. Next, the final volume of the mixed solutions were adjusted to 3000 μL with ultrapure water. The fluorescence emission spectra were recorded at an excitation wavelength of 334nm at room temperature.

Cell cytotoxicity assay

HaCaT cells were cultured in DMEM high glucose medium supplemented with

10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO_2 incubator. When cells were in logarithmic (Log) growth phase, they were seeded in a 96-well plate at a density of 10^3cells/well in 100 μL of culture medium. N-CDs were dissolved in culture medium and filtered through a 0.22 μm filter membrane aseptically. When HaCaT cells became confluent, 100 μL of N-CDs solution was added to the 96-well plate with the final concentration at 0, 200, 400, 600, 800, and 1000 μg/mL. Each condition was performed in quadruplicates. After 24 hrs incubation, the viability of HaCaT cells was determined using CCK-8 kit according to the manufacturer's instructions. Briefly, 20 μL of CCK8 solution was added to each well and the plate was incubated for 4 hours in the incubator. The absorbance was measured at 450 nm using a microplate reader and the cell viability was determined as follows: cell viability (%) = $(OD_{experimentalwell}/OD_{controlwell}) \times 100$.

Commercial metronidazole tablet analysis

To evaluate the availability of our prepared N-CDs for detecting commercial metronidazole samples, we purchased metronidazole tablets from a local pharmacy. A total of 100mg of the powder tablets was weighed accurately and dissolved in an appropriate amount of ultrapure water. The sample was sonicated for 20 min and adjusted to the final volume of 100 mL with ultrapure water. Each experiment was repeated three times (n=3) and the results were collected for further analysis.

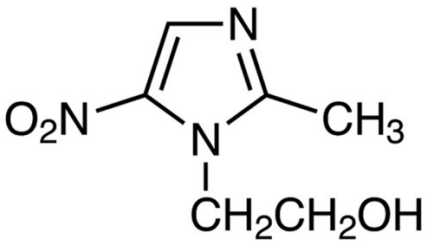

Figure S1 Molecular structure of metronidazole

Table S1 Fluorescence quantum yields of different reactant mass ratios

The mass ratio of reactants (g)	Fluorescence quantum yield (%)
Citric acid: urea=1:1	8.13
Citric acid: urea =1:2	16.86
Citric acid: urea =1:3	21.51
Citric acid: urea =1:4	21.18
Citric acid: urea =1:5	**26.71**

Table S2 Fluorescence quantum yields at different reaction temperatures

Reaction temperature (℃)	Fluorescence quantum yield (%)
210	27.81
200	27.12
190	**30.97**
170	13.48
160	26.88

Table S3 Fluorescence quantum yields at different reaction times

Reaction time(h)	Fluorescence quantum yield (%)
5	32.93
6	**35.08**
7	33.81
9	29.07
10	33.82

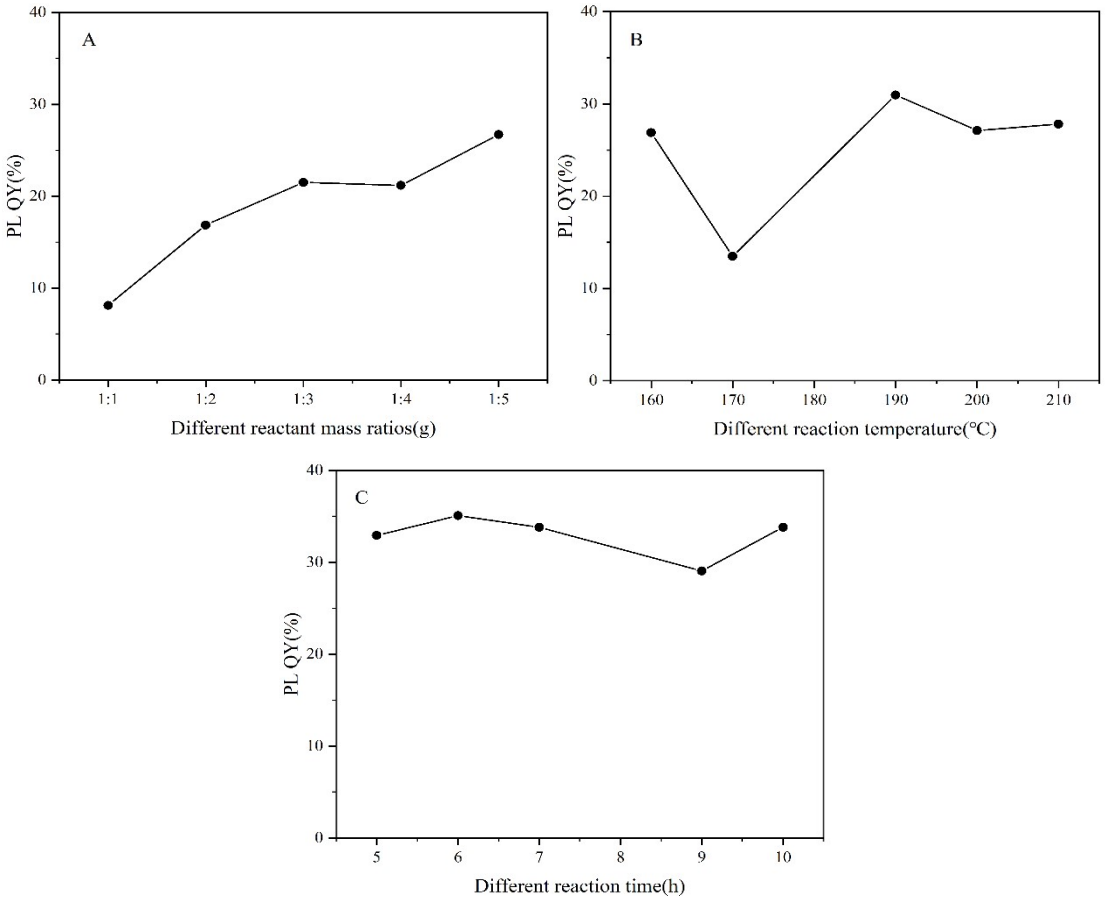

Figure S2 (A) Fluorescence quantum yields of different reactant mass ratios (B) Fluorescence quantum yields at different reaction temperatures (C) Fluorescence quantum yields at different reaction times

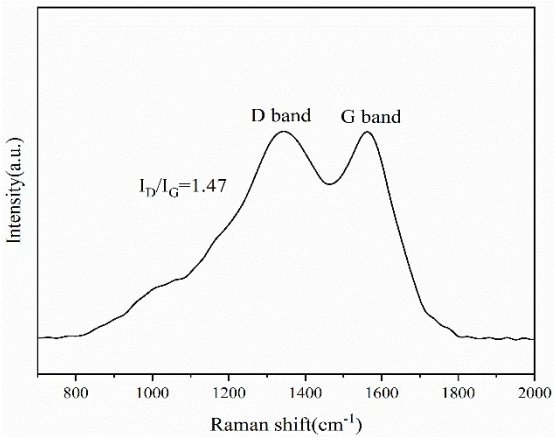

Figure S3 Raman spectrum of N-CDs