Electronic Supplementary Information

Determination of Folic acid with a Novel Biomass Sourced Carbon Quantum Dots-Mesoporous Silica Fluorescent Probe

Wen He,[*a,b] Soh Fong Lim,[a] Yan Gao,[b] Rubiyah Baini,[a] Jie Liu,[b] and Yingdi Zheng[b]

[a]Faculty of Engineering, Universiti Malaysia Sarawak, Kota Samarahan 94300, Sarawak, Malaysia.

E-mail: hewen1127@qlit.edu.cn.

[b] College of Biological and Chemical Engineering, Qilu Institute of Technology, Jinan, Shandong, 250200, China.

Supported by Research Program of Qilu Institute of Technology (No. QIT22NN004).

Experimental

Instruments

The instruments for this experiment is shown in Table S1.

Name	Model	Manufacturer
Drying Oven	DHG-9146A	Shanghai Jinghong Experimental Equipment Co., China
Centrifuges	H1850	Xiangyi Centrifuge Instrument Co. China
Magnetic Stirrer	SP-300	Hangzhou Mio Instrument Co., China
Water Bath	DF-101S	Gongyi Yingyu Gaoke Instrument Factory, China
UV-Vis Spectrophotometer	GENESYS 50	Thermo Fisher Scientific Co., US
Fluorescence Spectrophotometer	F-7000	Hitachi Ltd., Japan
Fourier transform infrared spectrometer spectrometer	Nicolet is5	Thermo Fisher Scientific Co. US
Freeze Dryer	SCIENTZ-10N/A	Ningbo Xinzhi Biotechnology Co., China
Transmission Electron Microscope	JEM-2100	Nippon Electronics Corporation, Japan
Malvern Laser Particle Sizer	Mastersizer 2000	Malvern Instruments Ltd., UK
X-ray Diffractometer	Ultima4	Nihon Rikaku Co., Japan
X-ray Photoelectron Spectroscopy	Escalab 250	Thermo Fisher Scientific Co. US
Fluorescence Spectrophotometer	FLS920	Edinburgh, UK
Specific Surface and Porosity Analyzer	Quadrasorb SI-MP	Kantar Instruments, USA
Zeta Potential Analyze	Zetasizer Nano ZS	Malvern Instruments Ltd., UK

Table S1 Experimental Instruments

Process optimization for CQDs-MSNs preparation

CQDs dosage. 0.015 g of MSN was taken in a round-bottom flask and added to 0.25 mL, 0.5 mL, 0.75 mL, 1.0 mL, 1.25 mL, 1.5 mL, 1.75 mL, and 2.0 mL of CQDs solution for mixing, and after magnetic stirring at 70°C for 30 min, the CQDs-MSN composite was obtained.

Reaction time. The CQDs-MSN composites were obtained by adding 0.015 g of mesoporous silica in a round-bottomed flask to 1.5 mL of CQDs solution for mixing, after magnetic stirring at 70°C for 10 min, 20 min, 30 min, 50 min, 60 min, 70 min, 80 min, and 90 min, respectively.

Reaction temperature. The CQDs-MSN composites were obtained by adding 0.015 g of mesoporous silica in a round-bottomed flask to 1.5 mL of CQDs solution for mixing, and stirring magnetically for 30 min at 30°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, and 110°C, respectively.

Characterization of CQDs-MSNs

Transmission electron microscopy (TEM). The CQDs solution and CQDs-MSN solution were first sonicated for 10 min and then dripped onto the ultrathin carbon film, which was dried and placed under the electron microscope to observe the morphology.

Scanning electron microscopy (SEM). 1mg CQDs-MSN powder was directly pasted on the conductive adhesive, and sprayed with gold using a coater for 45 s at a rate of 10 mA. The sample morphology and energy spectrograms were tested using a scanning electron microscope.

X-ray diffraction (XRD). firstly, the CQDs freeze-dried powder and CQDs-MSN powder were ground with mortar, and placed in the middle of the groove of the sample holder, so that the sample powder was slightly higher than the plane of the sample holder. Then the sample holder was inserted into the sample bay for testing.

Fourier transform infrared spectroscopy (FT-IR).After the KBr powder was well ground in an onyx bowl and pressed on a tablet press, the CQDs lyophilized powder and CQDs-MSN powder were well ground in an onyx bowl and pressed, and then the transmission spectra were tested by Fourier infrared spectrometer.

X-ray photoelectron spectroscopy (XPS). Appropriate amount of CQDs lyophilized powder and CQDs-MSN powder was pressed on the sample plate and put into the sample chamber for testing.

Ultraviolet-visible absorption spectroscopy (UV-vis). Firstly, 2 mL of ultrapure water and ethanol were taken into the cuvette for blank control, and then the CQDs solution and CQDs-MSN solution were put into the instrument for testing.

Fluorescence emission spectroscopy (FL). 2 mL of CQDs-MSN solution was put into a quartz cuvette for fluorescence detection.

Dynamic Light Scattering (DLS): 1 mL of CQDs solution, CQDs-MSN solution and CQDs-MSN+FA solution were ultrasonicated for 15 min and loaded into a four-way 25 transparent cuvette. The samples were placed into the chamber of the nano laser particle size analyzer for measurement. The scanning range was 0.1-1000 nm and the temperature was 25 °C. The dispersant was ultrapure water or ethanol. The measurement time of the sample was 60 s. The measurement was repeated three times and the average value was taken.

Nitrogen adsorption-desorption test (BET). The CQDs-MSN powder was weighed and prepared, and the sample tube containing CQDs-MSN powder was installed to the degassing station for degassing, and then the sample tube was transferred to the analyzing station for adsorption and desorption tests.

Fluorescence Lifetime Test (PL). The CQDs-MSN solution and the mixture of CQDs-MSN and FA were excited by laser light using a fluorescence spectrometer, and the excitation time was recorded to calculate the fluorescence lifetime.

Stability experiment

The stability of CQDs-MSN was examined by exposing the CQDs-MSN solution to UV irradiation for 30 h and repeating the fluorescence emission intensity every 5 h at λex=355 nm, λem=427 nm. Take 1 mL of NaCl solution with concentrations of 0 mM, 25 mM, 50 mM, 100 mM, 200 mM, 300 mM, 400 mM, 600 mM, 800 mM, 1000 mM and mix it with 500 μL of PBS buffer solution, respectively, and add it into 1 mL of CQDs-MSN solution, and then analyze the fluorescence spectra in different concentrations of NaCl solution at λex=355 nm, λem=427 nm. PBS (10 mmol/L) was selected as the buffer solvent in the experiment, and the pH values were 6.6, 6.8, 7.0, 7.2, 7.6, 7.8, and 8.0. 500 μL of PBS buffer solution was taken and added to 1 mL of CQDs-MSN solution for mixing, and the fluorescence intensity was measured at λex=355 nm, λem=427 nm. The changes in the fluorescence properties of biomass CQDs and CQDs-MSN in the long-term storage state were characterized by exposing the solutions to light irradiation for 30 d and repeating the fluorescence emission intensity every 5 days at λex=355 nm, λem=427 nm.

Feasibility experiments

500 μL of PBS buffer was added to 1000 μL of CQDs-MSN mixed solution, and then 500 μL of 0 μM, 25 μM, 50 μM FA solution was added, respectively. The fluorescence intensity was measured at λex=355 nm, λem=427 nm with a fluorescence spectrophotometer.

Optimization of conditions for detecting FA

Detection pH. Add 500 μL of PBS buffer to 1000 μL of CQDs-MSN mixing solution at pH 6.6, 6.8, 7.0, 7.2, 7.6, 7.8, 8.0, and then add 500 μL of 50 μM FA solution. The fluorescence intensity was measured at λex=355 nm, λem=427 nm with a fluorescence spectrophotometer, respectively.

Detection time. 500 μL of PBS buffer (pH 7.4) was added to 1000 μL of CQDs-MSN mixed solution, and then 500 μL of 50 μM FA solution was added. The fluorescence intensity was measured at λex=355 nm, λem=427 nm by fluorescence spectrophotometer at intervals during the reaction for 30 min, respectively.

Results and discussion

Optimization of experimental conditions for the preparation of CQDs-MSN

The volume of CQDs is an important factor affecting the fluorescence intensity of CQDs-MSN. As shown in Fig.S1a, the fluorescence intensity of CQDs-MSN at the optimal emission wavelength was gradually enhanced with the increase of carbon quantum dots addition, and the best fluorescence effect was achieved when 1.5 mL CQDs were added. This is because, when the volume of CQDs is too small, it leads to insufficient CQDs reaction and low fluorescence intensity[1]. And when there are too many CQDs, the strong attraction between nanoparticles caused by the high surface energy of CQDs may also contribute to the aggregation, and the aggregation of nanoparticles affects their fluorescence intensity[2]. Therefore, the optimal volume of CQDs is 1.5 mL.

The reaction time is one of the important influencing factors for the synthesis of CQDs-MSN. As shown in Fig.S1b, within a certain range, the longer the reaction time, the more fully the CQDs reacted and the fluorescence intensity was gradually enhanced. When the reaction time increases, the fluorescence intensity first increases and then decreases. This is because a short reaction time

leads to incomplete carbonization of CQDs, and a long reaction time causes further growth of the carbon nuclei of CQDs and is accompanied by other by-products, which affects the quantum yield of CQDs[3]. Therefore, the optimal reaction time for CQDs-MSN was 30 min.

The reaction temperature is one of the important factors affecting CQDs-MSN. As shown in Fig.S1c, the fluorescence intensity is strongest when the reaction temperature is 70 °C, and higher or lower temperatures decrease the fluorescence intensity. This is because the CQDs reaction becomes more and more sufficient as the temperature increases, and when the reaction temperature is too low, the energy provided is lower than that required for the n-π* jump to produce CQDs sufficiently[4]. While more CQDs will be generated at high temperatures, the associated aggregation of too many CQDs and the formation of crosslinked polymers on the surface of CQDs will lead to fluorescence burst. In order to prepare CQDs with longer fluorescence lifetimes, the aggregation of CQDs must be reduced[5]. Therefore, the optimal reaction temperature for CQDs-MSN is 70°C.

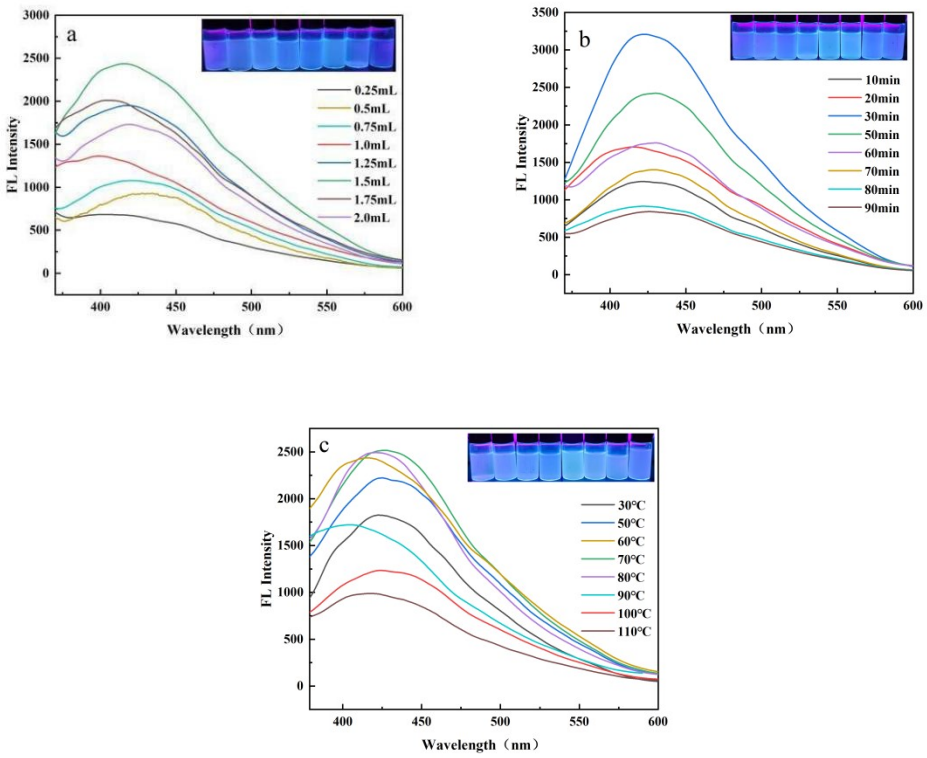

Fig. S1 Effect of CQDs-MSN reaction conditions on fluorescence intensity

(a) CQDs dosage (b) reaction time (c) reaction temperature

Reaction condition: (a) MSN 0.015g, CQDs 0.25mL, 0.50mL, 0.75mL, 1.0mL, 1.25 mL, 1.50 mL, 1.75mL, 2.0mL, Temperature=70°C, time=30min (b) MSN 0.015g, CQDs 1.5mL, Temperature=70°C, time=10min, 20min, 30min, 50min, 60min, 70min, 80min, 90min (c) MSN 0.015g, CQDs 1.5mL, Temperature =30°C, 50°C, 60°C, 70°C, 80°C, 90°C, 1000°C, 110°C, time = 30min.

Long-term storage stability experiment

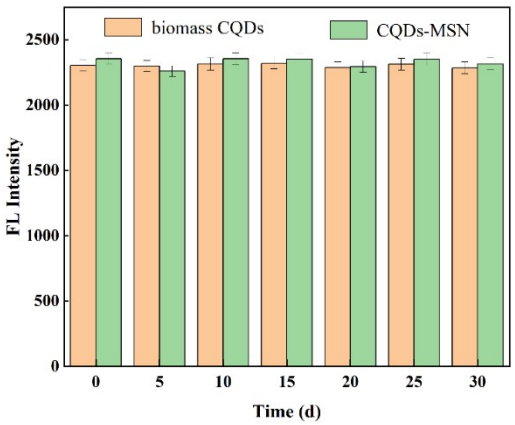

Fig. S2 Effect of light irradiation time on the fluorescence intensity of biomass CQDs and CQDs-MSN

Design principles

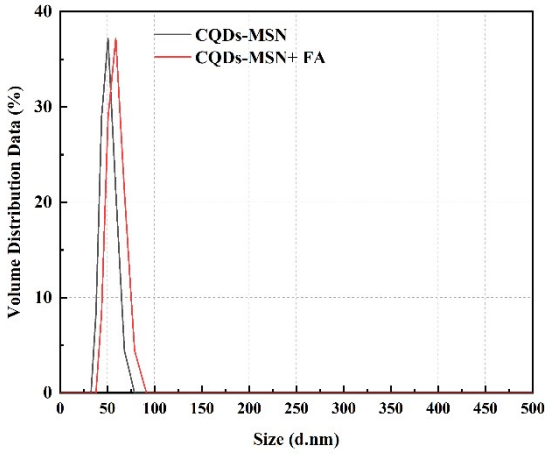

Fig.S3 Dynamic light scattering particle size distribution of CQDs-MSN before

and after adding FA

As shown in Fig.S3, upon adding FA to the CQDs-MSN solution, the particle size of CQDs-MSN increases from the original 50 nm to 58 nm. This is because when FA combines with the amino groups on the surface of CQDs to form amide bonds, it is equivalent to attaching new molecular groups (folic acid molecules) to the CQDs-MSN structure. These newly connected molecules increase the spatial volume of the system, thereby leading to an increase in particle size. In addition, the complex after combination may undergo a certain degree of aggregation, which may also increase the particle size.

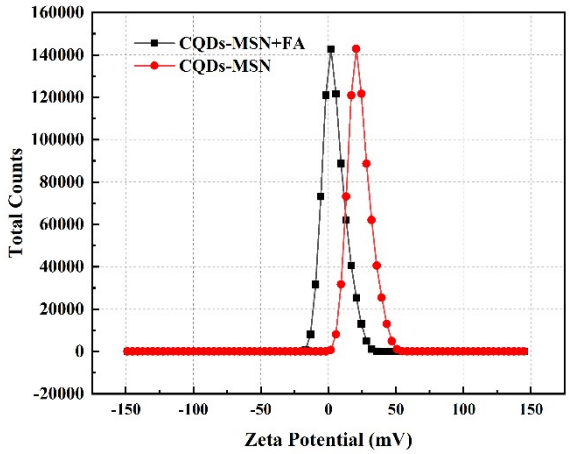

Fig.S4 Zeta potential of CQDs-MSN before and after adding FA

As shown in Fig.S4, due to the presence of amino groups on the surface of CQDs, the zeta potential of CQDs-MSN was 20.7 mV. After adding FA, since FA contains carboxyl groups which can be deprotonated and carry negative charges under certain conditions. Therefore, when the amino groups on the surface of CQDs combine with the carboxyl groups in FA to form amide bonds, the overall positive charge of the CQDs-MSN+FA system decreases and the negative charge relatively increases, resulting in a decrease in potential to 1.8 mV.

Feasibility experiments

The response of the CQDs-MSN fluorescent probe to FA was tested using CQDs-MSN fluorescent probe. The fluorescence spectrum of the CQDs-MSN nanoprobe is shown in Fig. S5.The probe has

a strong fluorescence emission peak at 427 nm at an excitation wavelength of 355 nm. The intensity of the fluorescence peak decreased significantly after the addition of a low concentration of FA solution, and decreased again after the addition of a high concentration of FA solution. The results of the above fluorescence spectroscopy experiments indicate the feasibility of the scheme of using CQDs-MSN nanoprobes to detect FA solution.

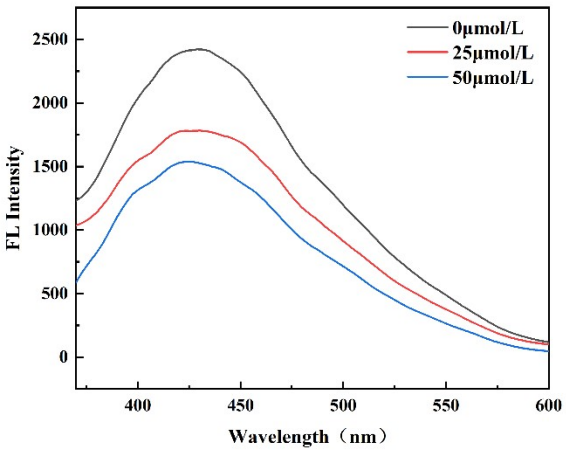

Fig.S5 Feasibility of CQDs-MSN for FA detection

(Reaction condition: λex=355 nm, λem=427 nm, slit 5.0 nm, emission spectral range 355-600 nm)

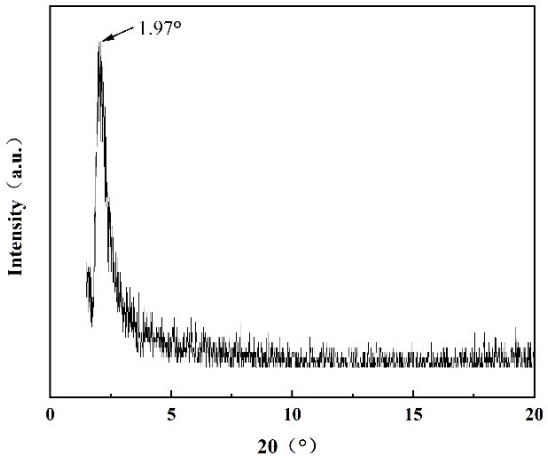

Fig.S6 XRD spectra of CQDs-MSN (1-20°)

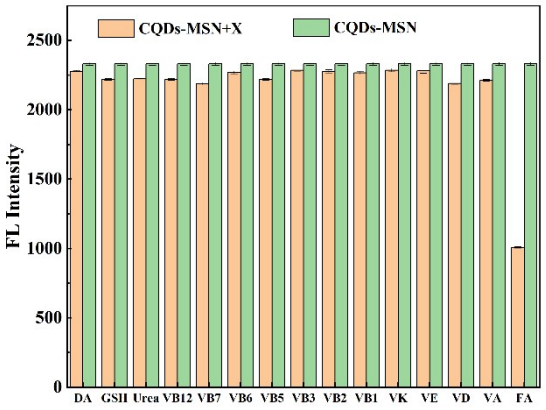

Fig.S7 Changes in the fluorescence of the CQDs-MSN towards other potential interfering substances (vitamins and substances contained in urine and serum) at the concentration of FA is 10 times lower

Fluorescence quenching mechanism

The Stern-Volmer equation was used to simulate the quenching effect[6].

$$F_0/F=1+c\times K\text{sv}=1+ c\times K\text{q}\times\tau_0 \quad (1)$$

where F_0 and F mean the fluorescence intensities at 427 nm with and without FA. Ksv is defined as the quenching constant ofthe equation. F_0/F and c represent the fluorescence quenching degree and FA concentration, respectively. Kq is the rate constant of the bimolecular quenching process. τ_0 is the fluorescence lifetime of the fluorescence probe in the absence of FA. As shown in Fig.S8, the Stern-Volmer curve was a curve that deflected upward from the axis towards the y-axis at high concentrations, indicating that the fluorescence quenching of FA involved both static quenching and dynamic quenching. Because if there was only one quenching method, the Stern-Volmer curve should have been linear[7]. In addition, in the low concentration region, it could be seen that the Stern-Volmer curve was linear, so Ksv and Kq (Table S1) could be calculated. As could be seen from the table, the value of Ksv gradually became smaller as the temperature increased, and the Kq value at all temperatures was much greater than the maximum diffusion collision quenching rate constant 2×10^{10} L/mol/s, indicating that the quenching of CQDs-MSN by FA was mainly static quenching.

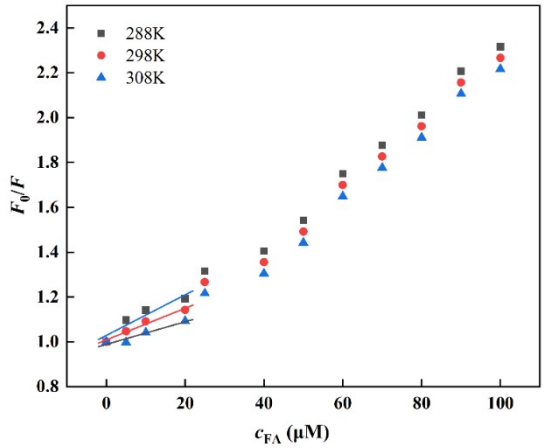

Fig.S8 Stern-Volmer curve of CQDs-MSN

Table S1 Stern-Volmer quenching constants, bimolecular quenching rate constants of CQDs-MSN induced by FA

T/K	*K*sv/(10^4 L/mol)	*K*q/(10^{12} L/mol/s)	R^2
288	0.85	4.9	0.9904
298	0.70	4.1	0.9976
308	0.45	2.6	0.9943

References

1 S. Aydin, A. Yilmaz and M. Yilmaz, *ChemistrySelect*, 2024, **9**(17), e202304930.

2 N. Saafie, N. S. Sambudi, M. D. H. Wirzal, and S., Sufian, *Separations*, 2023, **10**(2), 137.

3 J. X. Zheng, Y. T. Xie, Y. Y. Wei, Y. Z. Yang, X. G. Liu, Y. K. Chen, and B. S. Xu, *Nanomaterials*, 2020, **10**(1), 82.

4 Z. Q. Luo, C. Wu, M. Yan, X., Yu, X. M. Yu, Q. Qian, Y. T. Zhou, H. Zhang, Z. H. Li, and G. K. Long, *Materials Chemistry and Physics*, 2024, **316**, 129056.

5 L. Lin, Y. Yin, Z. Li, H. Xu, V. Hessel and K. K. Ostrikov, *Chemical Engineering Research and Design*, 2022, **178**, 395.

6 B. Limburg, *The Journal of Physical Chemistry Letters*, 2024, **15**, 10495-10499.

7 X. Wu and H. Li, *Microchemical Journal*, 2024, **201**, 110573.