Advances in Analytic Science (2023) Volume 4 Issue 1
doi: 10.54517/aas.v4i1.1946

Asia Pacific Academy of Science Pte. Ltd.

ORIGINAL RESEARCH ARTICLE

Fluorescence-based detection of anthrax biomarkers using copper-doped carbon nanodots

Peng Hua, Yu Huang, Yang Zhou, Fanyi He, Qionghui Yang, Weimei Zhu*

The Third People's Hospital of Yunnan Province, Kunming 650011, China

*** Corresponding author:** Wei-mei Zhu, 851907074@qq.com

ABSTRACT

In this study, water-soluble copper-doped carbon nanodots (Cu-CDs) with high fluorescence quantum yield were synthesized using a one-step hydrothermal process with citric acid and ethylenediamine as precursors and copper sulfate as the dopant. A novel fluorescence-based method for detecting anthrax biomarkers was developed, leveraging the strong chelation of 2,6-pyridinedicarboxylic acid (DPA) with the carbon nanodots. Under optimal conditions, the fluorescence quenching rate of Cu-CDs with DPA exhibited excellent linearity in the concentration ranges of 5–100 nmol/L ($r^2 = 0.9941$) and 150–400 nmol/L ($r^2 = 0.9976$), with a detection limit of 2.3 nmol/L. The method's low cost, high specificity, sensitivity, and simplicity suggest promising potential for anthrax biomarker detection.

Keywords: copper-doped carbon nanodots; fluorescent probes; anthrax biomarker; 2, 6-pyridin- edicarboxylic acid

Bacterial spore is a kind of dormant body formed in the spore of Bacillus, which has strong tolerance to the environment. Anthrax spore is a dangerous pathogen of Bacillus that can cause anthrax. Human beings can be infected by eating or inhaling anthrax spores, contacting infected animals with skin, or inhaling substances contaminated by bacteria[1,2]. Bacillus anthracis has been a serious threat to human life and health as a biological weapon, so accurate and sensitive detection of Bacillus anthracis is very important for preventing and controlling biological attacks and disease outbreaks[3,4]. 2, 6-pyridinic acid (DPA) is a unique component in anthrax spores, accounting for about 5%–15% of the dry weight of spores, which can be determined by spore physics. Release by chemical cracking or germination. Therefore, DPA can be used as an important biomarker in Bacillus anthracis for the detection of anthrax spores[5,6]. In recent years, the optical determination method of Bacillus anthracis mainly uses the fluorescence sensor of lanthanide metal elements to detect DPA[7,8]. However, the cost of lanthanide metal elements is high, and the fluorescence colorimetry is not obvious when the concentration of DPA is low. Therefore, the development of low-cost and high sensitivity. High selectivity. DPA detection method with low detection limit is still a research hotspot.

Fluorescent carbon nanodots (CDS) are monodisperse spherical nanoparticles with a size of less than 10 nm, with carbon as the basic skeleton and a large number of oxygen-containing groups on the surface[9,10]. Compared with traditional organic dyes and semiconductor quantum dots, fluorescent carbon nanodots not only have stable optical properties. It has the advantages of easy surface functionalization and large-scale preparation, and also has good biocompatibility and low cytotoxicity[11,12]. It can be used for in vivo and in

ARTICLE INFO
Received: 11 February 2023 | Accepted: 28 March 2023 | Available online: 10 April 2023

CITATION
Peng H, Yu H, Yang Z, et al. Fluorescence-based detection of anthrax biomarkers using copper-doped carbon nanodots. *Advances in Analytic Science* 2023; 4(1): 1946. doi: 10.54517/aas.v4i1.1946

vitro biological imaging. Fluorescent labeling. Drug delivery. Fluorescent probes and other fields have broad application prospects[13–15]. In recent years, there have been a lot of studies on the synthesis of doped carbon nanodots. Research reports on properties and applications, in which metal doping can greatly change the charge density of carbon nanodots. Optical and physicochemical properties, thereby expanding its application range[16,17].

Citric acid was used in this study. Using ethylenediamine as precursor and copper sulfate as metal dopant, a stable water-soluble blue luminescent copper doped carbon nanodots (Cu cds) was prepared by one-step hydrothermal method. Based on the strong chelation between DPA molecule and copper ion under acidic conditions, the specific recognition and detection of DPA by Cu cds was realized. This method is simple to operate. High sensitivity. It has low cost and can be used for the determination of anthrax biomarkers.

1. Experimental part

1.1. Instruments and reagents

G9800A fluorescence spectrophotometer (Agilent company of the United States), TECNAI G^2 TF30 field emission transmission electron microscope (Fei company of the Netherlands), X-ray photoelectron spectrometer (XPS, Thermo Fisher technology company), TENSOR27 Fourier infrared spectrometer (Bruker company of Germany), uv-2600 UV-Vis spectrophotometer (Shimadzu company of Japan), scilogex-MX-S vortex mixer (Beijing Kaiyuan Guochuang Technology Co., Ltd.), 120 Ml polytetrafluoroethylene (PTFE) lined hydrothermal reactor (Gongyi Yuhua Instrument Co., Ltd.), electric constant temperature blast drying oven (Shanghai Jinghong Experimental Equipment Co., Ltd.).

2, 6-pyridinedicarboxylic acid standard (DPA, purity >99%) was purchased from sigma Aldrich (Shanghai) Co., Ltd; benzoic acid. Organic small molecules such as phthalic acid and inorganic salt interferents such as ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were purchased from Shanghai Aladdin Reagent Co., Ltd; ethylenediamine. Citric acid and disodium hydrogen phosphate (Na_2HPO_4) and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. All chemicals and reagents used are analytically pure, and the experimental water is ultra pure water.

1.2. Experimental method

1.2.1. Preparation of copper doped carbon nanodots (Cu-CDs)

Accurately weigh 3.0 g citric acid and 2.0 g copper sulfate and dissolve them in 97 mL of ultra pure water, ultrasonic for 10 min to form a light blue solution, then add 3 mL ethylene diamine and stir evenly, transfer the solution to a polytetrafluoroethylene lined hydrothermal reactor, heat it at a constant temperature of 180 ℃ for 5 h, and naturally cool it to room temperature after the reaction is completed to obtain a brown solution. Pass the resulting solution over 0.22 μm filter membrane to remove large particle impurities, and then dialysis with a dialysis bag with a molecular weight of 3500 D for 24 h to obtain water-soluble copper doped carbon nanoparticles (Cu cds), which are stored at 4 ℃ for standby.

1.2.2. Determination of quantum yield

After the carbon nanodots are prepared into a solution, the UV-Vis absorption spectrum and fluorescence spectrum are tested (λ_{ex} = 350 nm). The fluorescence intensity value I and the corresponding absorbance a(λ_{em} = 440 nm) into the formula $\Phi_x = \Phi_R(n_x/n_R)^2(A_R/A_x)(I_x/I_R)$. Where, x is the test sample and R is the reference material; Φ Represents the fluorescence quantum efficiency, and n represents the refractive index of the solvent. Quinine sulfate (fluorescence quantum efficiency of 54%) was used as the standard in the experiment[18].

1.2.3. Determination method

Weigh an appropriate amount of DPA, dissolve it with ultra pure water, and prepare DPA solutions with different concentrations. Dilute the above prepared Cu-CDs to 2 mg/L, take 1 mL of Cu-CDs diluent into a 5 mL centrifuge tube, and add DPA stock solution 100 of different concentrationsμ50. Dilute to 3 mLwith disodium hydrogen phosphate citric acid buffer (50 mmol/L) with pH 6.0, vortex and mix well, and stand for 2 min at the excitation wavelength λ_{ex} = 348 nm, emission wavelength λ_{em} = measure the fluorescence intensity if and the reagent blank solution fluorescence intensity I_{F0} at 442 nm.

2. Results and discussion

2.1. Synthesis and characterization of carbon quantum dots

In this experiment, water-soluble Cu cds with high fluorescence quantum yield were prepared by one-step hydrothermal method using copper sulfate as the precursor of copper doping. **Figure 1A** shows the transmission electron microscope (TEM) of Cu cds. From the figure, it can be seen that the prepared carbon nanoparticles have a spherical like structure, good dispersion, uniform particle size, and an average size of about 2–3 nm.

Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS) were used to characterize the functional groups on the surface of carbon nanoparticles. **Figure 1B** shows the FI-IR spectrum of Cu-CDs. The peaks in the area of 3500–2700 cm^{-1} belong to the stretching vibration of O–H and N–H, and the peaks at 16811429 cm^{-1} belong to the stretching vibration of –COOH and o==c-nh, respectively, at 1162. The peak at 1044 cm^{-1} is attributed to the telescopic vibration of C–C or C==C, and the peak at 624 cm^{-1} is attributed to the telescopic vibration of C-Cu. In the XPS full spectrum of Cu-CDs (**Figure 1C**), 295.1. 404.2. 539.4. The four characteristic peaks at 944.2 EV are C1s respectively. N1s. O1s and cu2p. **Figure 1D** shows the high-resolution XPS spectrum of cu_2p. It can be seen from the figure that the synthesized carbon nanoparticles are mainly composed of carbon. Oxygen. Composed of nitrogen and divalent copper, copper doped carbon nanodots were successfully synthesized. In conclusion, there are a lot of amino groups on the surface of Cu cds. Carboxyl groups and other hydrophilic functional groups enhance the water solubility and stability of Cu-CDs.

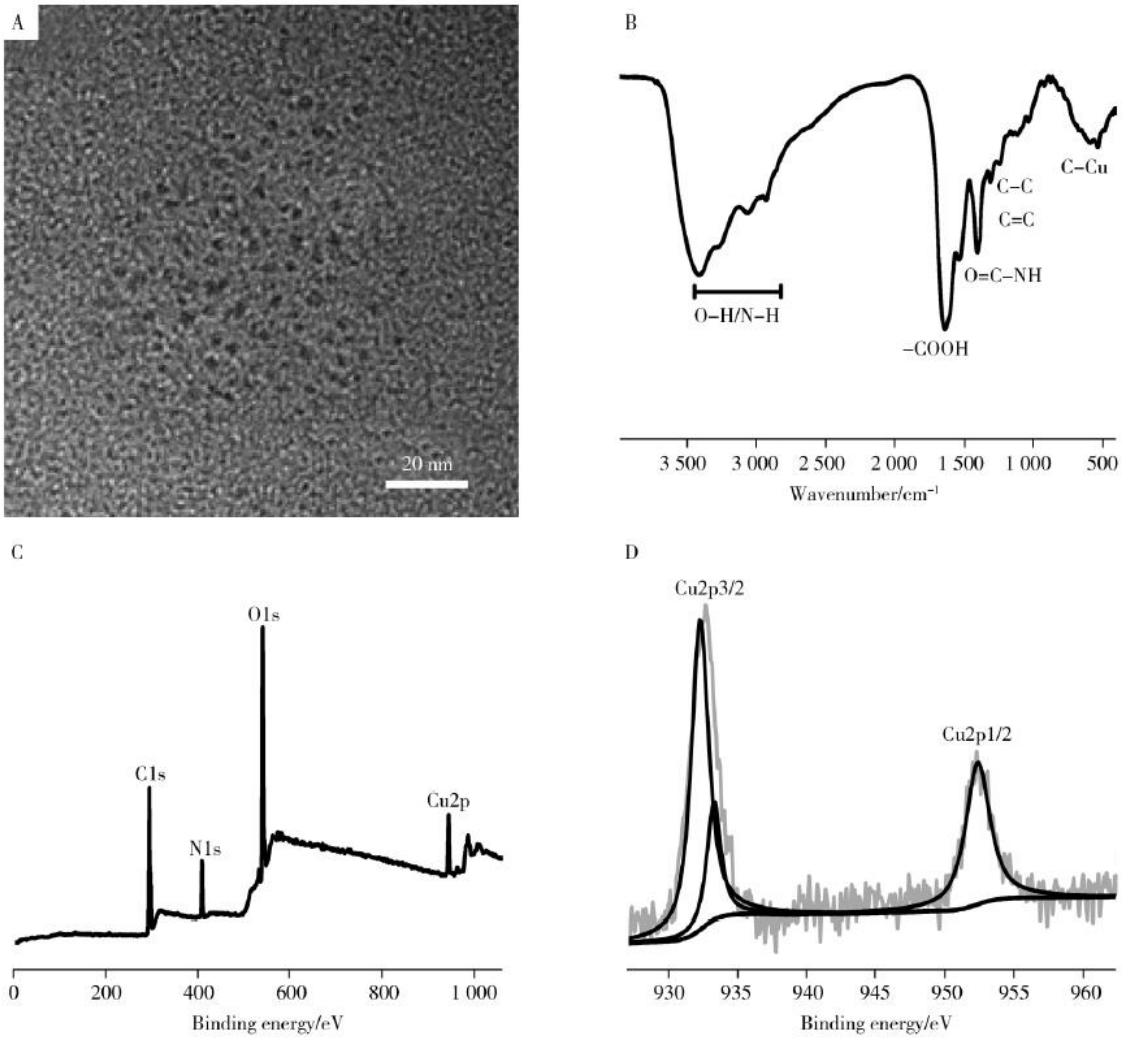

Figure 1. TEM image **(A)**, FT-irspectrum **(B)**, XPS spectrum **(C)** of Cu-CDs and Cu2p high-resolution XPS spectrum **(D)**.

Figure 2A shows the UV visible absorption and fluorescence excitation/emission spectra of Cu cds. In the UV visible absorption spectra, the absorption peaks at 234 nm and 342 nm are the characteristic peaks of Cu cds, the absorption peak at 234 nm is formed by the π - π^* transition of conjugated C==C, and the absorption peak at 342 nm is formed by the n - π^* transition of C==O. Under the maximum excitation wavelength of 348 nm, the maximum emission wavelength of Cu-CDs is 442 nm. Cu-CDs aqueous solution is transparent light yellow under natural light, while it is bright blue under ultraviolet light (365 nm) (illustration of **Figure 2B**). **Figure 2B** shows the emission spectra of Cu-CDs at different excitation wavelengths. When the excitation wavelength increases from 310 nm to 348 nm, the fluorescence intensity of Cu-CDs gradually increases, reaching the peak at 348 nm, and then the fluorescence intensity gradually decreases with the increase of excitation wavelength, and the position of emission peak does not change significantly between 310 and 390 nm. Using quinine sulfate (yield 54%, 0.1 mol/L H_2SO_4) as the reference material, the yield of fluorescent quantum dots of Cu cds was calculated to be 39.17%.

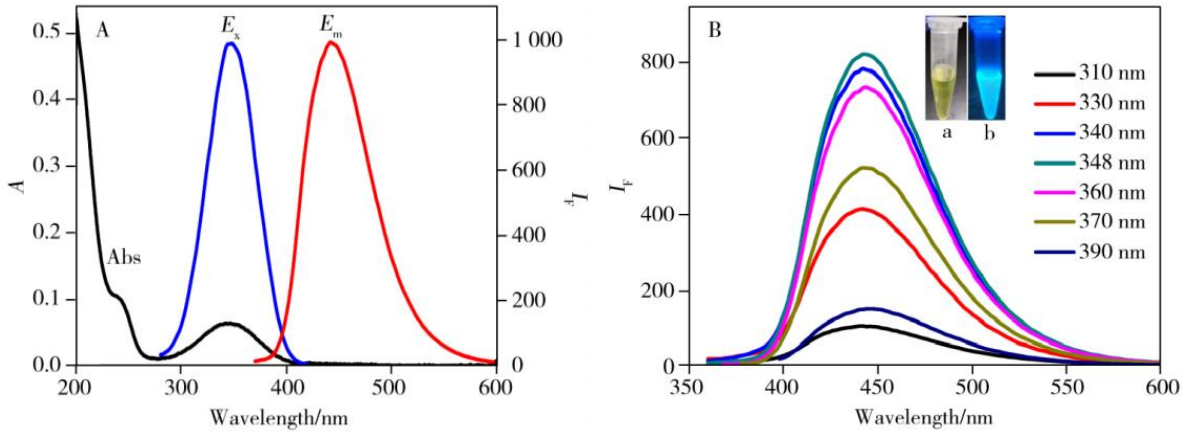

Figure 2. UV-Vis absorption spectrum and excitation/emission spectra of Cu-CDs **(A)** and fluorescence emission spectra with different excitation wavelengths **(B)** inset: photographs of Cu-CDs in aqueous solution under visible light (a) and UV light (b, 365 nm).

2.2. Measurement principle

As shown in **Figure 3**, Cu cds contains a large number of copper doped ions, and the carboxyl group and pyridine nitrogen of DPA molecule are the action sites that bind copper ions. Under acidic conditions, this action site can produce strong chelation with copper ions. Therefore, in the acidic system, when DPA is added to the Cu-CDs solution, Cu-CDS can react with DPA molecules rapidly, and DPA molecules are stably bound to the surface of Cu-CDs through chelation with copper ions. This chelating system enhances the π - π * conjugation absorption of Cu-CDs, and changes the energy level transition in the fluorescence generation process. Under the same excitation wavelength, the fluorescence emission wavelength of the system has a red shift. Therefore, with the increase of DPA concentration, the fluorescence red shift phenomenon is gradually obvious, and the blue fluorescence of Cu-CDs will gradually weaken[19].

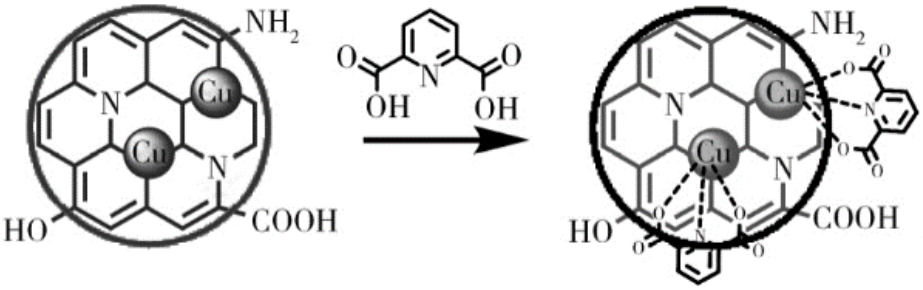

Figure 3. Schematic illustration of the Cu-CDs for DPA detection.

2.3. Optimization of reaction conditions

2.3.1. PH value

In this experiment, 50 mmol/L disodium hydrogen phosphate citric acid solution was selected as the buffer system, and the effect of pH value in the range of 2.0–8.0 on the fluorescence intensity of the system was investigated. It was found that pH 6.0 had the greatest effect on the change of fluorescence intensity of the system, so pH 6.0 was selected as the best reaction pH in this experiment.

2.3.2. Reaction time

The effect of reaction time on fluorescence quenching system was studied. The change value of fluorescence intensity of the system shall be measured every 1 min for 10 min. The results showed that the fluorescence intensity of the system increased gradually within 1–3 min, and then tended to be stable, so 3 min was the best reaction time.

2.4. Selectivity of the system to DPA

The selectivity of the quenching system to DPA was experimentally studied. As shown in **Figure 4**, under the same optimized experimental conditions, when the DPA concentration was 0.15 μmol/L, 40 times of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Cl^-, S^{2-}, NO_3^-, SO_4^{2-}, glucose, vitamin C, citric acid, sucrose, benzoic acid, phthalic acid, isophthalic acid, terephthalic acid, niacin, phenylalanine, cysteine Amino acid, tryptophan, serine, and the mixture of the above-mentioned different ions or organic small molecules did not interfere with the determination of DPA, indicating that the system has good selectivity for DPA.

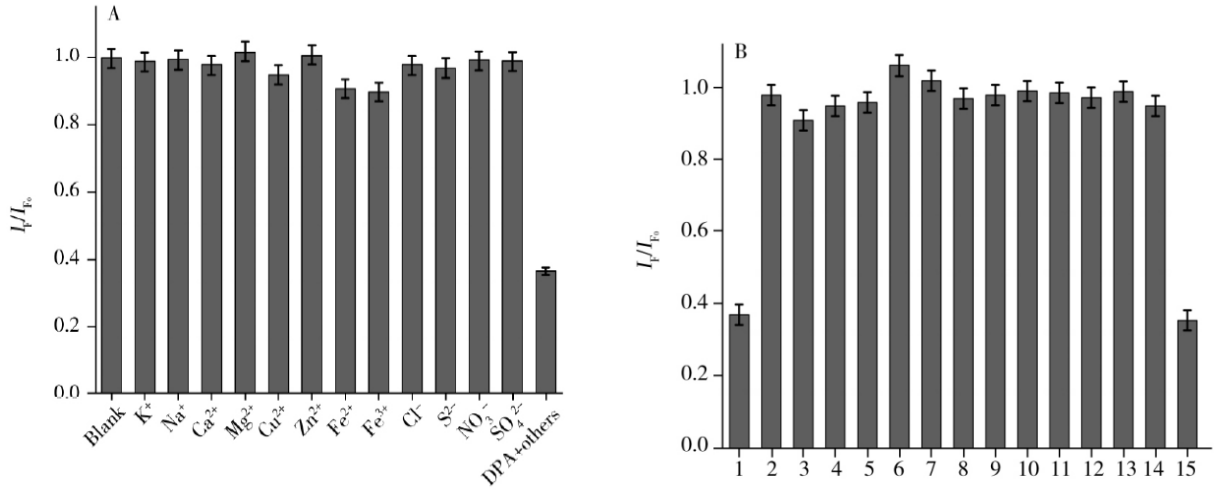

Figure 4. Selectivity of fluorescence system 1–15: zero point one fiveμmol/L DPA; sixμmol/L of glucose, vitamin C, citric acid, sucrose, benzoic acid, phthalic acid, isophthalic acid, terephthalic acid, nicotinic acid, phenylalanine, cysteine, tryptophan, serine, the mixture of 0.15 Mmol/L DPA with 6 Mmol/L of different organic small molecules, respectively.

2.5. System response to DPA

Under the optimized experimental conditions, the relationship between the fluorescence quenching degree of Cu cds and DPA concentration was investigated. As can be seen from **Figure 5A**, with the increase of DPA concentration, the fluorescence intensity of Cu-CDs gradually weakens and the spectrum gradually moves to the green wavelength. DPA concentration (c) has a linear relationship with Cu cds fluorescence quenching rate $(I_{F0} - I_F)/I_{F0}(y)$ in the range of 5–100 nmol/L and 150–400 nmol/L, respectively. The linear regression equations are $y = 0.0055c + 0.0016$ and $y = 0.0005c + 0.5723$, respectively. The correlation coefficients are $r^2 = 0.9941$ and $r^2 = 0.9976$, respectively, and the detection limit is 2.3 nmol/L (S/N = 3). As shown in **Figure 5B**, when different concentrations of DPA are added to the Cu-CDs solution, with the increase of DPA concentration, the color of the solution under 365 nm UV lamp gradually changes from blue to green, so the probe can be used for ratio determination.

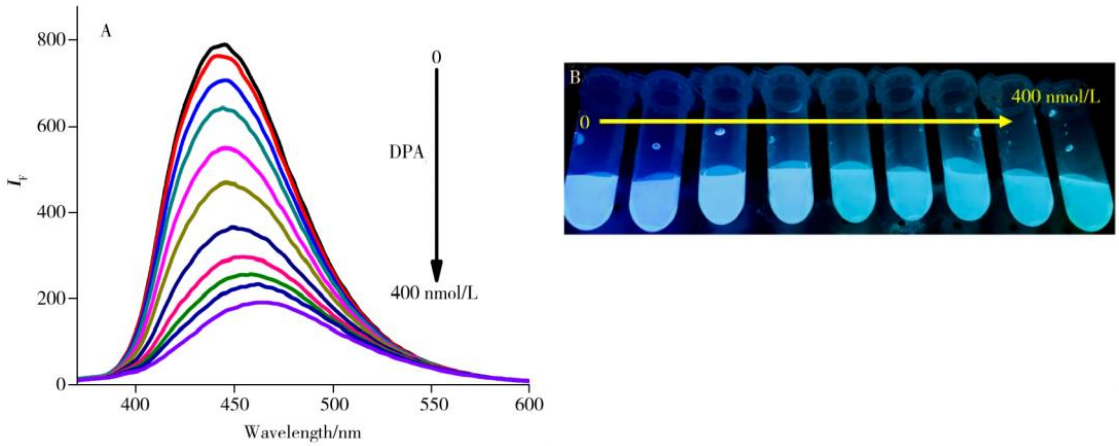

Figure 5. Fluorescence spectra (A) and aqueous solution colour under UV light (B) of Cu-CDs with different concentrations of DPA.

2.6. Determination of actual samples

Select the river. Tap water. Milk and calf serum are the analysis objects, and the water sample is first used with 0.22 μm filter membrane, and then centrifuge for 10 min at 15000 r/min to remove suspended large particle impurities; milk protein is removed by isoelectric point precipitation method, and the supernatant is taken for 0.22 μm filter membrane; 10% calf serum does not need to be treated. Each sample was determined in parallel for 5 times, and the results are shown in **Table 1**. The recovery rate of spiked standard is 95.2%–105%, RSD is 1.3%–3.8%.

Table 1. Analytical results for DPA in real samples by the proposed method (n = 5)

Samples	Added (nmol·L^{-1})	Found (nmol·L^{-1})	Recovery (%)	RSD (%)
River water	50	48.76	97.5	1.9
	150	154.93	103	3.8
	300	295.21	98.4	2.2
Tap water	50	49.54	99.1	3.4
	150	151.78	101	1.3
	300	289.82	96.6	2.5
Milk	50	47.62	95.2	2.9
	150	145.11	96.7	2.2
	300	311.46	104	3.7
Bovine serum	50	52.66	105	1.6
	150	156.16	104	2.1
	300	306.69	102	2.3

3. Conclusion

In this study, a high stability was prepared. Water soluble copper doped carbon nanodots (Cu-CDs) with high fluorescence quantum yield. Based on the strong chelation of anthrax spore biomarker 2, 6-pyridinedicarboxylic acid (DPA) and Cu_2 + in carbon nanodots, a new method for the determination of anthrax biomarkers by copper doped carbon nanodots ratio was established by quenching the blue fluorescence and changing the fluorescence color of Cu-CDs. Under the optimal conditions, the detection limit of the method reaches 2.3 nmol/L. This analytical method is highly exclusive. High sensitivity. The operation is simple and the cost is low, so it has a good application prospect.

Conflict of interest

The authors declare no conflict of interest.

References

1. Chen L, Fang Z. Modifying luminescent metal-organic frameworks with rhodamine dye: Aiming at the optical sensing of anthrax biomarker dipicolinic acid. Inorganica Chimica Acta. 2018; 477: 51-58. doi: 10.1016/j.ica.2018.02.032
2. Gao N, Zhang Y, Huang P, et al. Perturbing Tandem Energy Transfer in Luminescent Heterobinuclear Lanthanide Coordination Polymer Nanoparticles Enables Real-Time Monitoring of Release of the Anthrax Biomarker from Bacterial Spores. Analytical Chemistry. 2018; 90(11): 7004-7011. doi: 10.1021/acs.analchem.8b01365
3. Shi K, Yang Z, Dong L, et al. Dual channel detection for anthrax biomarker dipicolinic acid: The combination of an emission turn on probe and luminescent metal-organic frameworks. Sensors and Actuators B: Chemical. 2018; 266: 263-269. doi: 10.1016/j.snb.2018.03.128
4. Yilmaz MD, Oktem HA. Eriochrome Black T–Eu3+ Complex as a Ratiometric Colorimetric and Fluorescent Probe for the Detection of Dipicolinic Acid, a Biomarker of Bacterial Spores. Analytical Chemistry. 2018; 90(6): 4221-4225. doi: 10.1021/acs.analchem.8b00576
5. Li Y, Li X, Wang D, et al. Hydroxyapatite nanoparticle based fluorometric turn-on determination of dipicolinic acid, a biomarker of bacterial spores. Microchimica Acta. 2018; 185(9). doi: 10.1007/s00604-018-2978-0
6. Rong M, Liang Y, Zhao D, et al. A ratiometric fluorescence visual test paper for an anthrax biomarker based on functionalized manganese-doped carbon dots. Sensors and Actuators B: Chemical. 2018; 265: 498-505. doi: 10.1016/j.snb.2018.03.094
7. Wang QX, Xue SF, Chen ZH, et al. Dual lanthanide-doped complexes: the development of a time-resolved ratiometric fluorescent probe for anthrax biomarker and a paper-based visual sensor. Biosensors and Bioelectronics. 2017; 94: 388-393. doi: 10.1016/j.bios.2017.03.027
8. Donmez M, Oktem HA, Yilmaz MD. Ratiometric fluorescence detection of an anthrax biomarker with Eu3+-chelated chitosan biopolymers. Carbohydrate Polymers. 2018; 180: 226-230. doi: 10.1016/j.carbpol.2017.10.039
9. Li T, Tang JL, Fang F, et al. Carbon quantum dots: synthesis, properties and applications. J. Funct. Mater. 2015; 9(36): 9012-9025.
10. Zhang XF, Lu JB, Wang XM. Preparation, Fluorescence Properties and Cell Imaging Application of Thermo-sensitive Carbon Quantum Dots. J. Instrum. Anal. 2018; 37(2): 198–203.
11. Hou X, Hu Y, Wang P, et al. Modified facile synthesis for quantitatively fluorescent carbon dots. Carbon. 2017; 122: 389-394. doi: 10.1016/j.carbon.2017.06.093
12. Wang D, Zhang H, Guo J, et al. Biomimetic Gradient Polymers with Enhanced Damping Capacities. Macromolecular Rapid Communications. 2016; 37(7): 655-661. doi: 10.1002/marc.201500637
13. Zhu S, Meng Q, Wang L, et al. Highly Photoluminescent Carbon Dots for Multicolor Patterning, Sensors, and Bioimaging. Angewandte Chemie International Edition. 2013; 52(14): 3953-3957. doi: 10.1002/anie.201300519
14. Zhan XF, Tang JS, Wu J, Cao ZK. Determination of Copper Ions in Water Samples by Silicon Doped Carbon Quantum Dots. J. Instrum. Anal. 2016; 35(11): 1461-1465.
15. Peng Z, Han X, Li S, et al. Carbon dots: Biomacromolecule interaction, bioimaging and nanomedicine. Coordination Chemistry Reviews. 2017; 343: 256-277. doi: 10.1016/j.ccr.2017.06.001
16. Bera K, Sau A, Mondal P, et al. Metamorphosis of Ruthenium-Doped Carbon Dots: In Search of the Origin of Photoluminescence and Beyond. Chemistry of Materials. 2016; 28(20): 7404-7413. doi: 10.1021/acs.chemmater.6b03008
17. Lin L, Luo Y, Tsai P, et al. Metal ions doped carbon quantum dots: Synthesis, physicochemical properties, and their applications. TrAC Trends in Analytical Chemistry. 2018; 103: 87-101. doi: 10.1016/j.trac.2018.03.015
18. Devi P, Thakur A, Chopra S, et al. Ultrasensitive and Selective Sensing of Selenium Using Nitrogen-Rich Ligand Interfaced Carbon Quantum Dots. ACS Applied Materials & Interfaces. 2017; 9(15): 13448-13456. doi: 10.1021/acsami.7b00991
19. Li P, Ang AN, Feng H, et al. Rapid detection of an anthrax biomarker based on the recovered fluorescence of carbon dot–Cu(ii) systems. Journal of Materials Chemistry C. 2017; 5(28): 6962-6972. doi: 10.1039/c7tc01058c