A New Dual-peak Fluorescent Probe for Water Content Detection Made From Taxus

Gang Wang1 · Yaping Li1 · Haipeng Chen3 · Shuqin Tang1 · Yiyang Cheng3 · Yuhong Yu1 · Abdul Qayoom Majeedano1 · Shangrao Pu2 · Gang Wang1

Received: 17 March 2022 / Accepted: 27 May 2022 / Published online: 30 June 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
In this paper, the leaves of Taxus were used as the sole carbon source, and two kinds of carbon dots blue and red, with different properties, were synthesized by the hydrothermal method under different conditions. The red carbon dots were quenched in the water, and the blue carbon dots had stable fluorescence properties in water environment. The bimodal fluorescence probe formed by mixing could accurately and stably measure the water content in ethanol, which was in the range of 82.5%-100%, is highly correlated with the fluorescence intensity ratio \(I_{481}/I_{678}\) of mixed carbon dots under 390 nm excitation light, with \(R^2 = 0.995\) and the detection limit as low as 0.31%. The experimental materials are environmentally friendly, low in cost, and simple to operate, as well as the water content measured by proportional fluorescence has high accuracy, which provides a new method for measuring moisture in ethanol.

Keywords Fluorescent probe · Taxus · Water content · Dual-peak fluorescent probe

Introduction
As a fluorescent nano luminescent material, carbon dots are highly sensitive and specific [1–3], which has received extensive attention in recent years. The main synthesis methods of carbon are various, and the main synthesis method is the hydrothermal method [4, 5]. Solvothermal method [6, 7] and microwave-assisted method [8–11]. Carbon has excellent selectivity in detecting trace antibiotics [12–15], heavy metal ions [5, 16–18], Pesticides [19–23], and medical imaging [24–26]. The aspect plays a huge role compared with a unimodal fluorescent probe, bimodal fluorescent probe has a high resolution, high precision, and strong stability [27–29] advantages, etc. Dried leaves of Taxus are used as biological carbon sources, and two kinds of carbon dots (481 nm and 678 nm) are synthesized by the hydrothermal method respectively. The fluorescence of red carbon dots can be quenched by water. The disadvantages are weak luminescence, large error, weak

Shangrao Pu
psr@sicau.edu.cn

Gang Wang
wanggang@sicau.edu.cn
Gang Wang
james@stu.sicau.edu.cn
Yaping Li
2020304060@stu.sicau.edu.cn
Haipeng Chen
chengehaipeng@stu.sicau.edu.cn
Shuqin Tang
2021304097@stu.sicau.edu.cn
Yiyang Cheng
chengyiyang@stu.sicau.edu.cn

Yuhong Yu
2019204011@stu.sicau.edu.cn
Abdul Qayoom Majeedano
aqm@stu.sicau.edu.cn

1 College of Forestry, Sichuan Agricultural University, 611130 Chengdu, Sichuan province, China
2 Department of Landscape Plants, Sichuan Agricultural University, 611130 Chengdu, Sichuan province, China
3 College of Science, Sichuan Agricultural University, 625014 Ya’an, Sichuan province, China
sensitivity of blue carbon dots to water, and strong luminescence intensity. The dual-ratio fluorescence probe is formed by mixing two kinds of carbon dots. It can effectively make up for the shortcomings of the low sensitivity of blue carbon dots and weak luminescence of red carbon dots, enhance the stability of fluorescence data, and can be used for the detection of water content in ethanol.

Materials and Methods
Reagents

The leaves of Taxus were collected in the Taxus base of Fanzhuangya Garden Company, which was cooperated by the Dujiangyan Campus of Sichuan Agricultural University. Silica gel, petroleum ether, ethyl acetate, ethanol, and various metal salts were purchased from Shanghai McKinley Co., Ltd., and all chemical reagents in this work are analytical pure reagents, which can be used directly without further purification.

Instruments and Characterization

The fluorescence spectrophotometer (Hitachi, F-4500, Tokyo, Japan) recorded the fluorescence spectrum and analyzed the optical properties and related activities of the products. The characterization parameters (morphology, size, etc.) of two kinds of carbon dots were detected by a high-resolution transmission electron microscope (HTEM) (JEOL 2100 F, Japan). The content and structure of elements were revealed by spectra measured by ESCALAB 250Xi photoelectron spectrometer (Thermo Scientific, USA).

Results
Carbon Point Characterization

HTEM analysis of the red and blue carbon dots and their mixed carbon dots (Fig. 1) shows that the red carbon dots are clustered, with a size of 37.8 ~ 64.8 nm, the lattice width of 0.32 ~ 0.36 nm, the blue carbon dots are spherical, with the size of 2.7 ~ 4.1 nm and the lattice width of 0.33 ~ 0.45 nm. After the red and blue carbon dots were mixed, the blue carbon dots gathered around the red carbon dots to form a new aggregate, forming a dual-signal fluorescent solution (Fig. 2).
Optical Properties of Mixed Carbon Points

The ethanol solution of mixed carbon dots is pale yellow or yellow under natural light, which indicates that the prepared carbon dots have good solubility in organic solvents. The fluorescence spectra of blue carbon dots, red carbon dots, and their mixed carbon dots were studied at room temperature. Separately different excitation conditions study in blue carbon dots with fixed concentrations, and the excitation wavelength ranges from 360 to 410 nm, as shown in Fig. 3. When the excitation wavelength is 360 nm, a blue fluorescence peak was observed at about 450 nm. With the increase of the excitation wavelength, the fluorescence peak moves to the far-infrared wavelength end, and the

![Fig. 1 Synthesis of carbon dots and selectivity of mixed carbon dots to water](image)

![Fig. 2 HTEM diagram of red carbon dots (a), lattice electron microscope diagram of red carbon dots (b), blue carbon dots and lattice diagram (c), electron microscope diagram of mixed red and blue carbon dots (d)](image)
intensity of the fluorescence peak gradually decreases. The fluorescence spectra of red fluorescent carbon dots with different excitation wavelengths were studied separately. When the excitation wavelength was 360 nm, the red fluorescence peak was observed at about 678 nm. With the increase of excitation light wavelength, the fluorescence intensity gradually increased, and the fluorescence peak did not move. After the mixing of the blue-red carbon dot solution, two peaks were formed at 450 nm and 678 nm, which together form a dual emissivity w system for moisture detection. In order to determine the best mixing ratio of two kinds of carbon dots, 6 kinds of mixed carbon dot solutions were prepared and carried out for fluorescence detection. It can be seen from the figure that the blue band fluorescence intensity decreases with the increase of excitation wavelength, while the red band fluorescence intensity increases with the increase of excitation wavelength (Figs. 3 and 4). Finally, considering comprehensively, it is determined that the excitation wavelength is 390 nm, and the mixing ratio of blue and red carbon dots is 1:1, so as to complete the final construction of the dual-ratio fluorescent probe (Figs. 5 and 6).

**Sensitivity of H₂O**

The fluorescence quenching effect was observed by adding different water contents into a mixed carbon point ethanol solution (Fig. 7). The mixed fluorescence spectra showed that with the increase of water content in the sample, the fluorescence intensity at 481 nm remained unchanged, the quenching effect at 678 nm was obvious, and the fluorescence intensity decreased gradually. The ratio of fluorescence intensity \( \frac{I_{481}}{I_{678}} \) increased with the increase in water content. It shows that the fluorescence intensity ratio of mixed carbon dots \( \frac{I_{481}}{I_{678}} \) is highly correlated with water content. The fluorescence intensity ratio \( I_{481}/I_{678} \) and water content \( R^2 = 0.995 \) fit well in the range of 82.5%-100% (Fig. 8). The detection limit is obtained by the ratio of triple blank standard deviation to the curve slope, and its value is as low as 0.31%.

**Selectivity of Mixed Carbon Points**

In order to test the anti-interference performance of the dual-ratio fluorescent probe, in the presence of 8 kinds of metal ions with a concentration of 100 μM, the mixed solution of ethanol and carbon dots was excited at 390 nm, and the fluorescence intensity ratio \( \frac{I_{481}}{I_{678}} \) of the solution with only fluorescent mixed carbon dots was set as \( F_0 \), and the fluorescence intensity ratio \( \frac{I_{481}}{I_{678}} \) of other metal ions was set as \( F \). The \( F/F_0 \) produced by the quenching phenomenon was observed and calculated. These results show that even if there are excessive metal ions coexisting with the solution,
**Fig. 4** Fluorescence diagram of red carbon dots

**Fig. 5** Proportional mixed fluorescence diagram
Fig. 6 Fluorescence diagram of mixed carbon dots

Fig. 7 Water content quenching fluorescence diagram
these interferences can be ignored. The acute response of H$_2$O in ethanol solution to fluorescence quenching also shows that mixed carbon dots can still accurately identify H$_2$O in the presence of various metal ions (Fig. 9).

Fig. 8 Linear fitting diagram

![Linear fitting diagram](image)

\[ y = 47.1112x - 36.0377 \]
\[ R^2 = 0.9958 \]

Fig. 9 Heavy metal interference

![Heavy metal interference](image)
The Analysis of H2O with Standard Addition

In order to evaluate the practical application ability of this method, H2O was spiked and recovered, and three kinds of water contents 85%, 90%, and 95%, were spiked and recovered respectively, and then the spiked analysis table was made based on its fluorescence analysis. It can be seen from the Table 1 that the average recovery rate of H2O added in ethanol is 99.36%-100.96%, and the relative standard deviation is less than 1%, which can be used for quantitative analysis of samples.

## Conclusions

In this study, the branches and leaves of Taxus were used as raw materials, and two kinds of carbon dots with different properties were synthesized by the hydrothermal method. After mixing them, based on the quenching effect of H2O on the mixed carbon dots, a double-emission fluorescence system was prepared, and a new proportional fluorescence determination method of H2O was proposed. The developed method has a linear range of 82.5%-100% and a low detection limit of 0.31%. H2O in ethanol was determined. The result is satisfactory, which shows that the sensing system has good sensitivity, high selectivity, and effective feasibility.

## Acknowledgements

Authors thank supports from the Key Research and Development Projects of Sichuan Province and Sichuan Liangshan Science and Technology Plan Projects.

## Author Contributions

G W (student), YP L, HP C and YY C completed the experimental part and the writing part, SQ T and YH Y were responsible for the charts and review, A QM reviewed the whole paper and finished the revision of the English manuscript. SR P (professor) and G W (professor) were responsible for the whole experiment and the writing of the later paper. All authors read and approved the final manuscript.

## Funding

This research work is supported by the Key Research and Development Projects of Sichuan Province (2018NZ0097), Sichuan Liangshan Science and Technology Plan Projects (21ZDFY0152).

## Availability of Data and Material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethical Approval

Not applicable.

### Consent for Publication

Not applicable.

### Informed Consent

A statement regarding informed consent is not applicable.

### Conflict of Interest

All authors, including Gang Wang – Yaping Li – Haipeng Chen – Shuqin Tang – Yiyang Cheng – Yu Yuhong – Abdul Qayoom Majedeano – Shangrui Pu – Gang Wang, declare no conflict of interest.

## References

1. Xue H, Yu M, He K et al (2020) A novel colorimetric and fluorometric probe for biothiols based on MnO2 NPs-Rhodamine B system. Anal Chim Acta 1127:39–48. https://doi.org/10.1016/j.aca.2020.06.039

2. Sui L, Wang X, Chang Q et al (2019) Selective determination of acetone by carbon nanodots based on inner filter effect. Spectrochim Acta Part A Mol Biomol Spectrosc 216:290–295. https://doi.org/10.1016/j.saa.2019.03.059

3. Anju SM, Anjana RK, Vijila NS et al (2020) Tb-doped BSA–gold nanoclusters as a bimodal probe for the selective detection of TNT. Anal Bioanal Chem 412(17):4165–4172. https://doi.org/10.1007/s00216-020-02654-0

4. Egorova MN, Tomskaya AE, Kapitonov AN et al (2018) Hydrothermal synthesis of luminescent carbon dots from glucose and birch bark soot. J Struct Chem 59(4):780–785. https://doi.org/10.1134/S0022476618040054

5. Li DY, Wang SP, Azad F et al (2020) Single-step synthesis of polychromatic carbon quantum dots for macroscopic detection of Hg2+. Ecotoxicol Environ Saf 190:110141. https://doi.org/10.1016/j.ecoenv.2019.110141

6. Song T, Zhao Y, Wang T et al (2020) Carbon dots doped with N and S towards controlling emitting. J Fluoresc 30(1):81–89. https://doi.org/10.1007/s10895-019-02472-3

7. Li T, Shuang E, Wang J et al (2019) Regulating the properties of carbon dots via a solvent-involved molecule fusion strategy for improved sensing selectivity. Anal Chim Acta 1088:107–115. https://doi.org/10.1016/j.aca.2019.08.027

8. Bashir I, Staszewski RB, Balasra PT (2016) A digitally controlled injection-locked oscillator with fine frequency resolution. IEEE J Solid-State Circ 51(6):1347–1360. https://doi.org/10.1109/JSSC.2016.2539342

9. Rahimi M, Mahani M, Hassani Z (2019) Carbon quantum dots fluorescence quenching for potassium optode construction. Luminescence 34(4):402–406. https://doi.org/10.1002/bio.3634

10. Hagiwara K, Horikoshi S, Serpone N (2021) Luminiscent mono-dispersed carbon quantum dots by a microwave solvothermal method toward bioimaging applications. J Photochem Photobiol A 415:113310. https://doi.org/10.1016/j.jphotochem.2021.113310

11. Pajewska-Szymt M, Buszewski B, Gadzka-Kopciuch R (2019) Sulphur and nitrogen doped carbon dots synthesis by microwave assisted method as quantitative analytical nano-tool for mercury ion sensing. Mater Chem Phys 242:122484. https://doi.org/10.1016/j.matchemphys.2019.122484
12. Wang X, Chen C, Waterhouse GIN et al (2021) A novel SERS sensor for the ultrasensitive detection of kanamycin based on a Zn-doped carbon quantum dot catalytic switch controlled by nucleic acid aptamer and size-controlled gold nanorods. Food Chem 362:130261. https://doi.org/10.1016/j.foodchem.2021.130261

13. Jalili R, Irani-Nezhad MH, Khataee A et al (2021) A ratiometric fluorescence probe based on carbon dots and gold nanocluster encapsulated metal–organic framework for detection of cephalixin residues in milk. Spectrochim Acta Part A Mol Biomol Spectrosc 262:120089. https://doi.org/10.1016/j.saa.2021.120089

14. Gao Z, Liu M, Xu K et al (2020) Facile fabrication of N/S/P tri-doped carbon dots for tetracycline detection by an internal filtering effect of a two-way matching strategy. Anal Methods 12(20):2551–2554. https://doi.org/10.1039/D0AY00249F

15. Narimani S, Samadi N (2021) Rapid trace analysis of ceftriaxone using new fluorescent carbon dots as a highly sensitive turn-off nanoprobe. Microchem J 168:106372. https://doi.org/10.1016/j.microc.2021.106372

16. Zhang Y, Li C, Sun L et al (2021) Defects coordination triggers red-shifted photoluminescence in carbon dots and their application in ratiometric Cr(VI) sensing. Microchem J 169:106552. https://doi.org/10.1016/j.microc.2021.106552

17. Sheng X, Li S, Zhan Y et al (2021) Selective detection of Cu^{2+} using nitrogen-doped carbon dots derived from humic acid and urea based on specific inner filter effect. Spectrochim Acta Part A Mol Biomol Spectrosc 263:120136. https://doi.org/10.1016/j.saa.2021.120136

18. Cai L, Fu Z, Cui F (2020) Synthesis of carbon dots and their application as turn off–on fluorescent sensor for mercury (II) and glutathione. J Fluoresc 30(1):1–20. https://doi.org/10.1007/s10895-019-02454-5

19. Wang S, Chen H, Xie H et al (2021) A novel thioctic acid-carbon dots fluorescence sensor for the detection of Hg^{2+} and thiophanate methyl via S-Hg affinity. Food Chem 346:128923. https://doi.org/10.1016/j.foodchem.2020.128923

20. Zhu X, Yuan X, Han L et al (2021) A smartphone-integrated optical sensing platform based on red-emission carbon dots for real-time detection of pyrethroids. Biosens Bioelectron 191:113460. https://doi.org/10.1016/j.bios.2021.113460

21. Gogoi J, Chowdhury D (2020) Calcium-modified carbon dots derived from polyethylene glycol: fluorescence-based detection of Trifluralin herbicide. J Mater Sci 55(25):11597–11608. https://doi.org/10.1007/s10853-020-04839-5

22. Khaledian S, Noroozi-Aghideh A, Kahrizi D et al (2021) Rapid detection of diazinon as an organophosphorus poison in real samples using fluorescence carbon dots. Inorg Chem Commun 130:108676. https://doi.org/10.1016/j.inoche.2021.108676

23. Yadav M, Das M, Bhatt S et al (2021) Rapid selective optical detection of sulfur containing agrochemicals and amino acid by functionalized cyclodextrin polymer derived gold nanoprobes. Microchem J 169:106630. https://doi.org/10.1016/j.microc.2021.106630

24. Li C, Xu Y, Liu S et al (2021) Cancer cell identification by facile imaging of intracellular reductive substances with fluorescent nanosensor. Talanta 234:122650. https://doi.org/10.1016/j.talanta.2021.122650

25. Alexandre MR, Costa AI, Berberan-Santos MN et al (2020) Finding value in wastewaters from the cork industry: carbon dots synthesis and fluorescence for hemeprotein detection. Molecules 25(10):2320. https://doi.org/10.3390/molecules25102320

26. Chang D, Zhao Z, Niu W et al (2021) Iron ion sensing and in vitro and in vivo imaging based on bright blue-fluorescent carbon dots. Spectrochim Acta Part A Mol Biomol Spectrosc 260:119964. https://doi.org/10.1016/j.saa.2021.119964

27. Liu Y, Wu M, Zhu L et al (2015) Colorimetric and fluorescent bimodal ratiometric probes for pH sensing of living cells. Chem Asian J 10(6):1304–1310. https://doi.org/10.1002/asia.201500106

28. Brand C, Abdel-Atti D, Zhang Y et al (2014) in vivo imaging of GLP-1R with a targeted bimodal PET/fluorescence imaging agent. Bioconjug Chem 25(7):1323–1330. https://doi.org/10.1021/bc50178d

29. Tamil Selvan G, Varadaraju C, Tamil Selvan R et al (2018) On/off fluorescent chemosensor for selective detection of divalent iron and copper ions: molecular logic operation and protein binding. ACS Omega 3(7):7985–7992. https://doi.org/10.1021/acsomega.8b00748

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.