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

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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 hydrothermal method under different conditions. The red carbon dots were quenched in 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 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, simple to operate, and the water content measured by proportional fluorescence has high accuracy, which is provides a new method for measuring moisture in ethanol.

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 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 [16–19], Pesticides [20–24], and medical imaging [25–27]. The aspect plays a huge role compared with unimodal fluorescent probe, bimodal fluorescent probe has high resolution, high precision and strong stability [28–30] advantages, etc. Dried leaves of *Taxus* are used as biological carbon source, and two kinds of carbon dots (481 nm and 678 nm) are synthesized by hydrothermal method respectively. The fluorescence of red carbon dots can be quenched by water. The disadvantages are weak luminescence, large error, weak sensitivity of blue carbon dots to water, but strong luminescence intensity. The dual-ratio fluorescence probe formed by mixing two kinds of carbon dots, It can effectively make up for the shortcomings of 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 Fanghua Garden Company, which was cooperated by 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

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 high resolution transmission electron

microscope (HRTEM) (JEOL 2100 F, Japan). The content and structure of elements were revealed by spectra measured by ESCALAB 250Xi photoelectron spectrometer (Thermo Scientific, USA).

The influence of H_2O content on the fluorescence spectrum of mixed carbon dots in ethanol system

1) Fresh leaves of *Taxus* were drying in an oven (60℃, 6 hours), then cutt into small pieces for later use, firstly synthesizing red carbon dots, weighing 1 g of dried leaves, put them in a reaction kettle, 20 ml of absolute ethanol added, left for reaction in the oven at 120℃ for 5 hours, after the reaction kettle naturally cooled, absorbed the reaction solution, filtered through polyethersulfone membrane, and obtained filtrate carrried out silica gel column chromatography. The eluent mixed solution of petroleum ether and ethyl acetate with a volume ratio of 1:1, and the obtained chromatographic solution was concentrated by rotary evaporation, and the concentrated solution was transferred to a blank test tube (the blank test tube was weighted in advance), dried in an oven at 65℃ for 24 hours, then the test tube was weighted again, and the quality of red carbon dots was obtained by differential calculation.

2) Synthesizing blue carbon dots, 1g of dried *Taxus* leaves were weighted and put them in a reaction kettle, 20 ml of deionized water was added, reaction in an oven at 180℃ for 5h, sucking the reaction solution after the reaction kettle was cooled, filtered with polyethersulfone membrane, concentrated the obtained filtrate by rotary evaporation, concentrated solution was transferred to a blank test tube (the blank test tube was weighted in advance). After dried in an oven at 65℃ for 24 hours, the test tube was weighted again, and the quality of red carbon dots was obtained by differential calculation.

3) Mixing the synthesis and fluorescence spectra of carbon dots, red carbon dots was diluted and blue carbon dots with ethanol to 100 mg/ml respectively, and mixed them to form a mixed solution, setting a series of ethanol-water mixed solutions with equal water content gradient (2.5%) in the range of 82.5%-100% water content, 1 ml of red was mixed and blue carbon dots mixed solution and 1ml of ethanol-water mixed solution with a total volume of 2 ml,The fluorescence spectra of 481nm and 678 nm were recorded at the excitation wavelength of 390 nm, and the fluorescence intensity ratio (I_{481}/I_{678}) at 481 nm and 678 nm was calculated. At the same time, 1 mM different kinds of metal ion solutions were prepared, 100 μl of metal ion solutions were mixed with 900 μl of absolute ethyl alcohol, and 1 ml of red, blue and carbon dots solutions were added, totaling 2 ml. The fluorescence spectra were recorded at the excitation wavelength of 390 nm and the fluorescence intensity ratio (I_{481}/I_{678}) at 481 nm and 678 nm was calculated.By changing the water content in the ethanol-water mixed solution, the mixed carbon point sensing system can finally selectively detect the water content in ethanol.

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 the 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.

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 of blue carbon dots with fixed concentration, and the excitation wavelength ranges from 360 nm to 410 nm, as shown in the Fig. 2.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 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 moved. After the mixing of blue-red carbon dot solution two peaks were formed at 450 nm and 678 nm, which together form a dual emissivity fluorescence 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 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. 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.

Sensitivity of H_2O

The fluorescence quenching effect was observed by adding different water contents into mixed carbon point ethanol solution (Fig. 5). 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 (I_{481}/I_{678}) increased with the increase of water content. It shows that the fluorescence intensity ratio of mixed carbon dots (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. 6). The detection limit is obtained by the ratio of triple blank standard deviation to 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 (I_{481}/I_{678}) of the solution with only fluorescent mixed carbon dots was set as F_0, and the fluorescence intensity ratio (I_{481}/I_{678}) of other metal ions was set as followsThe F_0/F produced by the quenching phenomenon was observed and calculated. These results show that even if there are excessive metal ions coexisting with the solution, these interferences can be ignored. The acute response of H_2O in ethanol solution to fluorescence quenching also shows that mixed carbon dots can still accurately identify H_2O in the presence of various metal ions.

The analysis of H_2O with standard addition

In order to evaluate the practical application ability of this method, H_2O was spiked and recovered, and three kinds of water contents of 85%, 90%, 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 H_2O 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.

Table 1
Sample recovery table

H_2O added(ml)	H_2O found(ml)	Recovery(%)	RSD(%)
	0.8470	99.65%	
0.85	0.8475	99.71%	0.32%
	0.8531	100.36%	
	0.8984	99.82%	
0.9	0.8946	99.40%	0.21%
	0.8988	99.86%	
	0.9494	99.74%	
0.95	0.9545	100.96%	0.27%
	0.9488	99.36%	

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 hydrothermal method. After mixing them, based on the quenching effect of H_2O on the mixed carbon dots, a double emission fluorescence system was prepared, and a new proportional fluorescence determination method of H_2O was proposed. The developed method has a linear range of 82.5%-100% and a low detection limit of 0.31%. H_2O in ethanol was determined,The

result is satisfactory, which shows that the sensing system has good sensitivity, high selectivity and effective feasibility.

Declarations

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Contributions:

G W(student), YP L, HP C and YY C jointly completed the experimental part and the writing part, SQ T and YH Y were responsible for the charts and review, QM A 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.

Corresponding author:

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Ethical Approval:

Not applicable.

Consent for publication

Not applicable.

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.

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 – Qayoom Majeedano Abdul – Shangrao Pu – Gang Wang, declare no conflict of interest.

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Figures

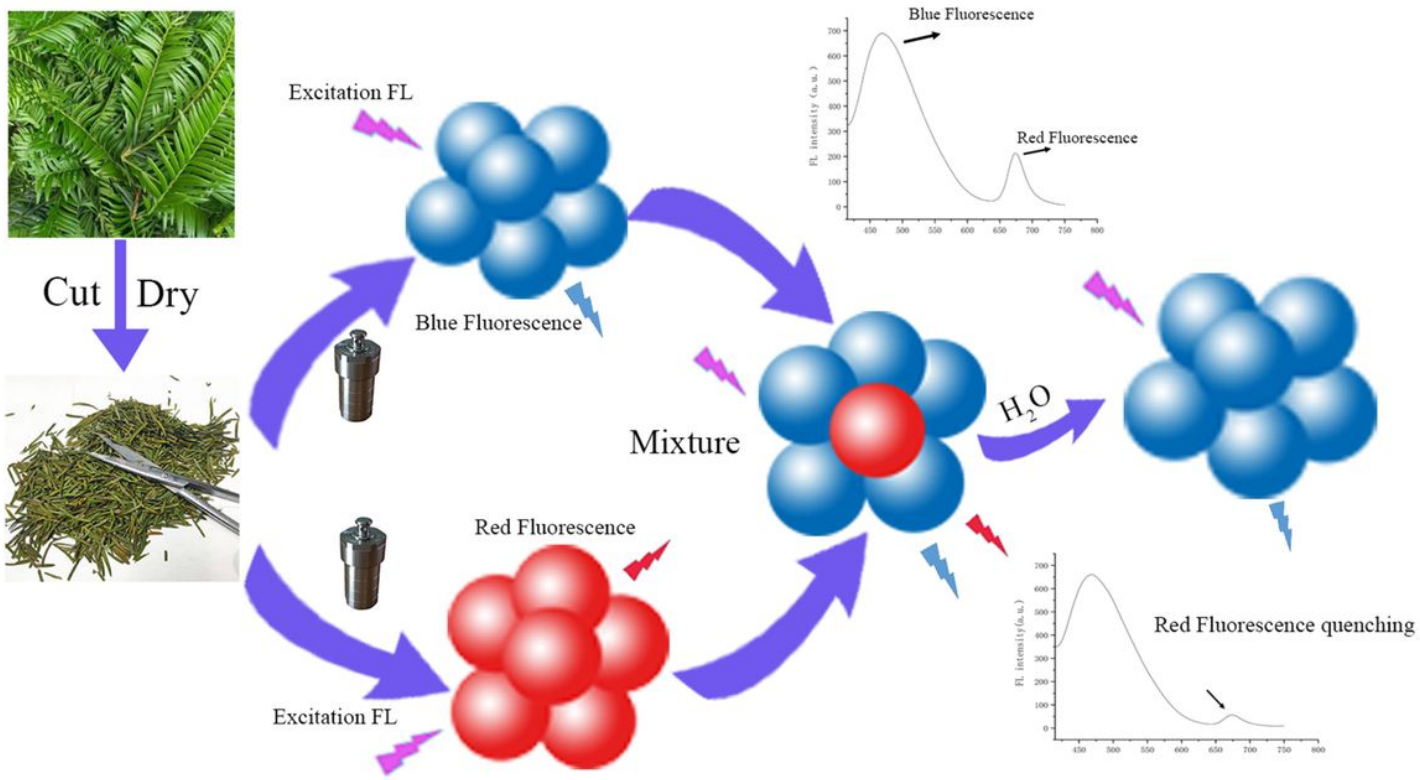

Figure 1

Synthesis of carbon dots and selectivity of mixed carbon dots to water

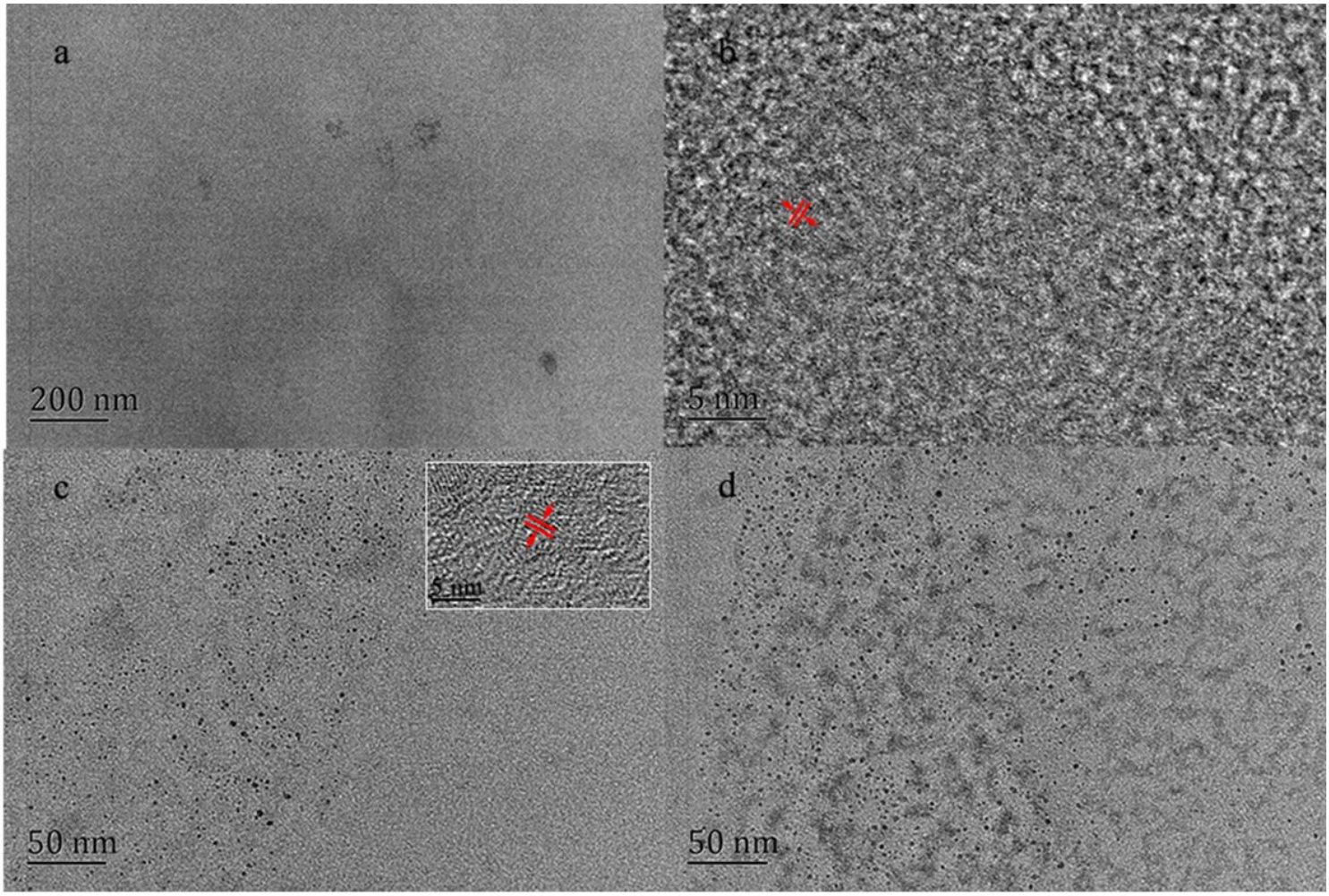

Figure 2

Electron microscope 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)

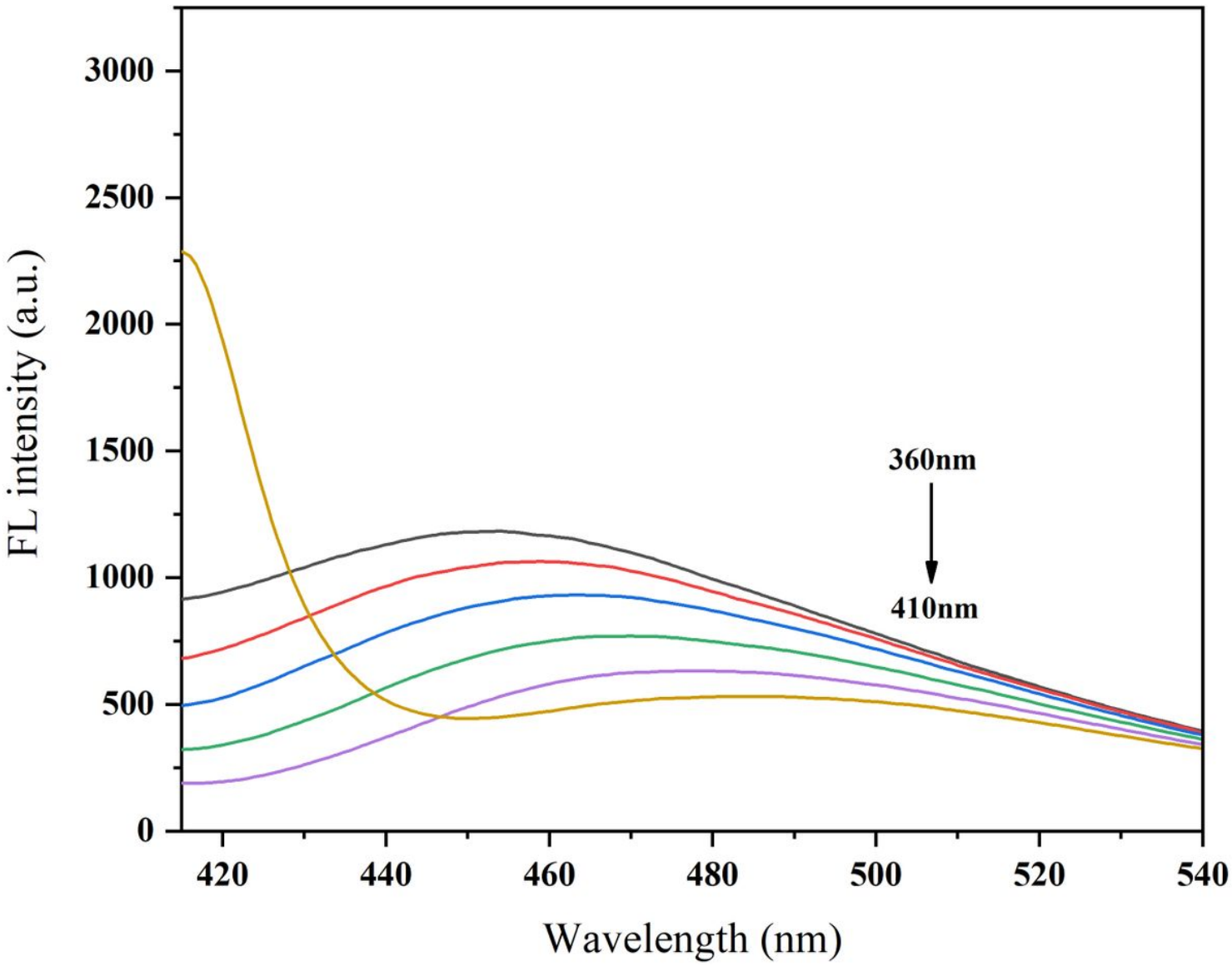

Figure 3

Fluorescence diagram of blue carbon dots

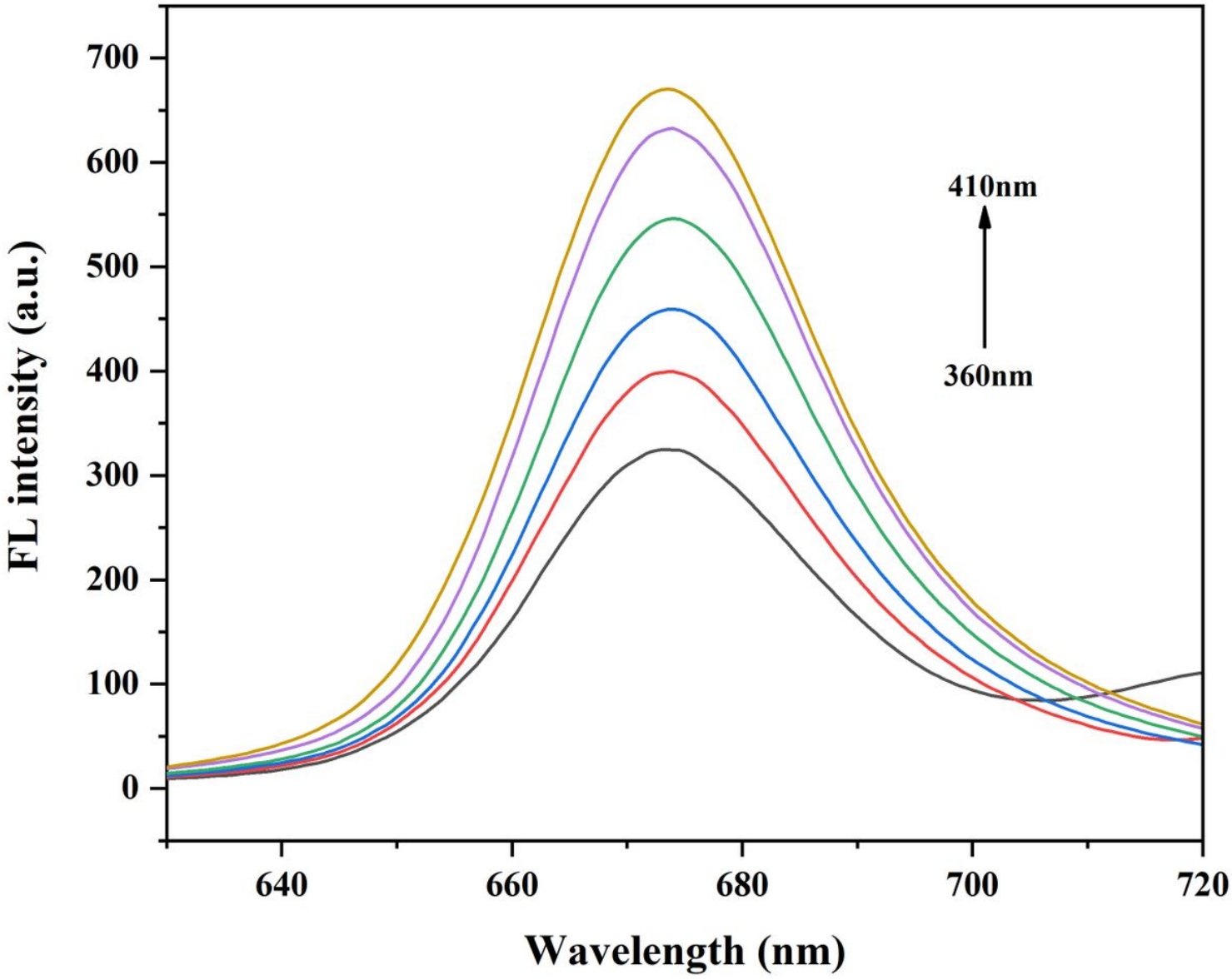

Figure 4

Fluorescence diagram of red carbon dots

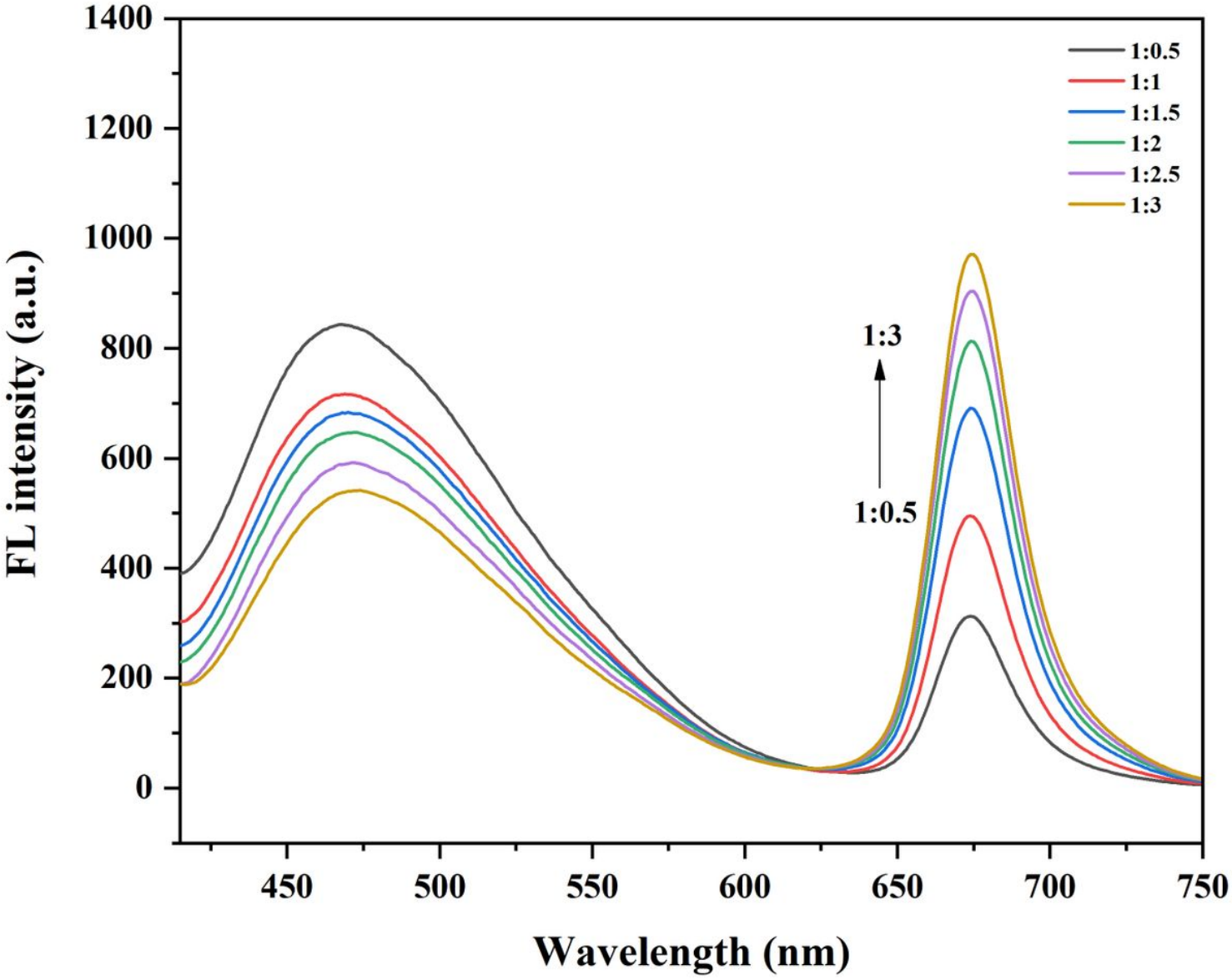

Figure 5

Proportional mixed fluorescence diagram

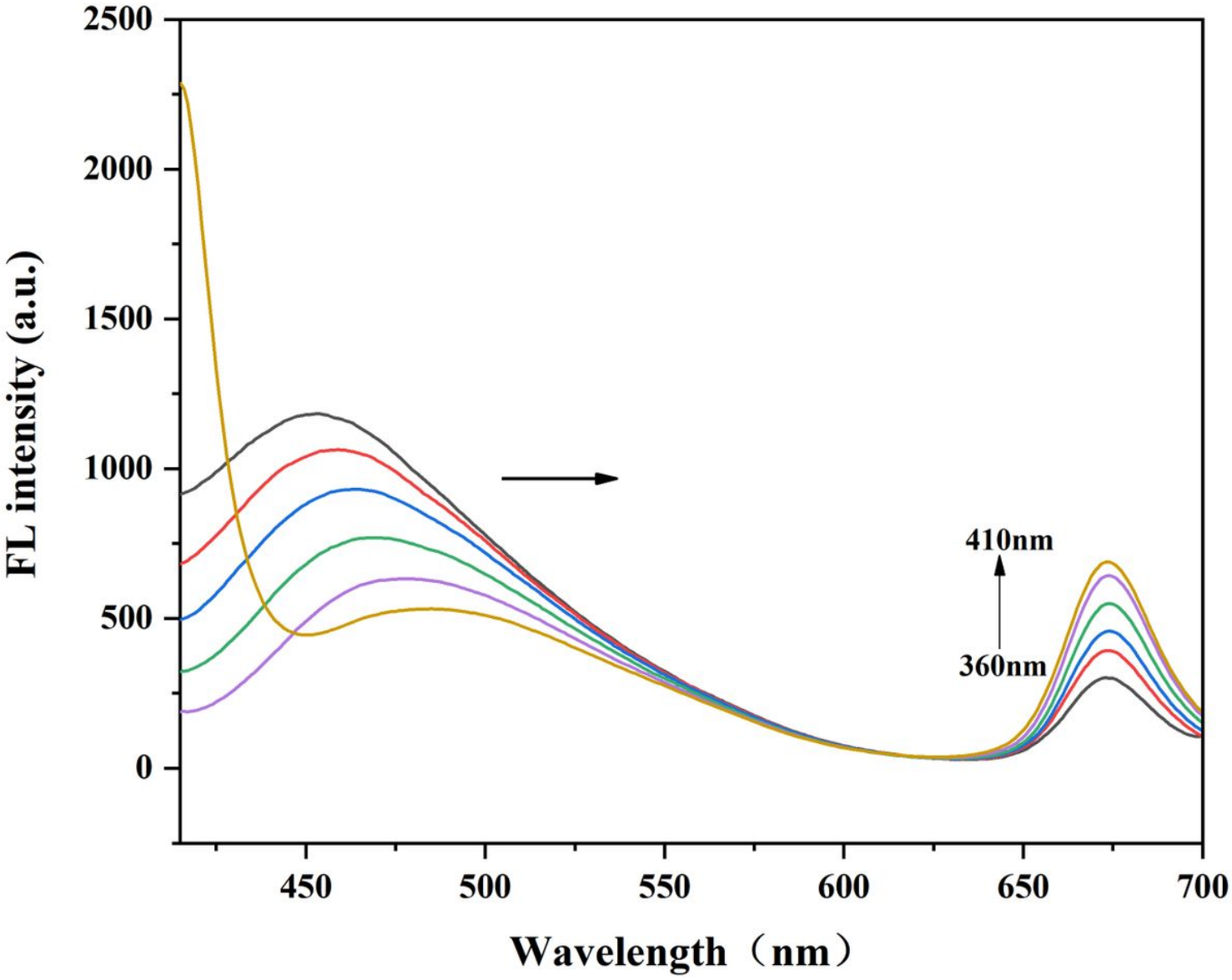

Figure 6

Fluorescence diagram of mixed carbon dots

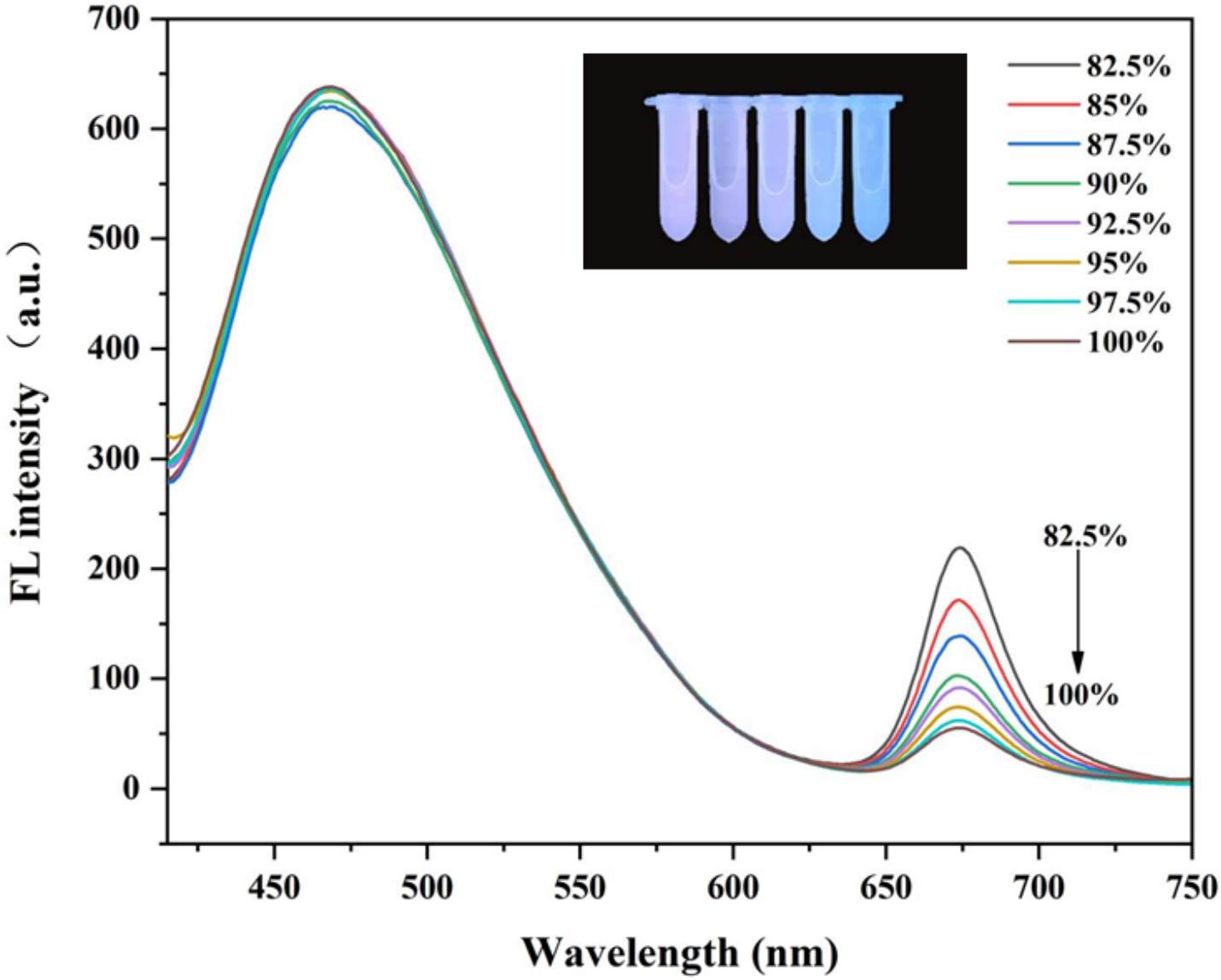

Figure 7

Water content quenching fluorescence diagram

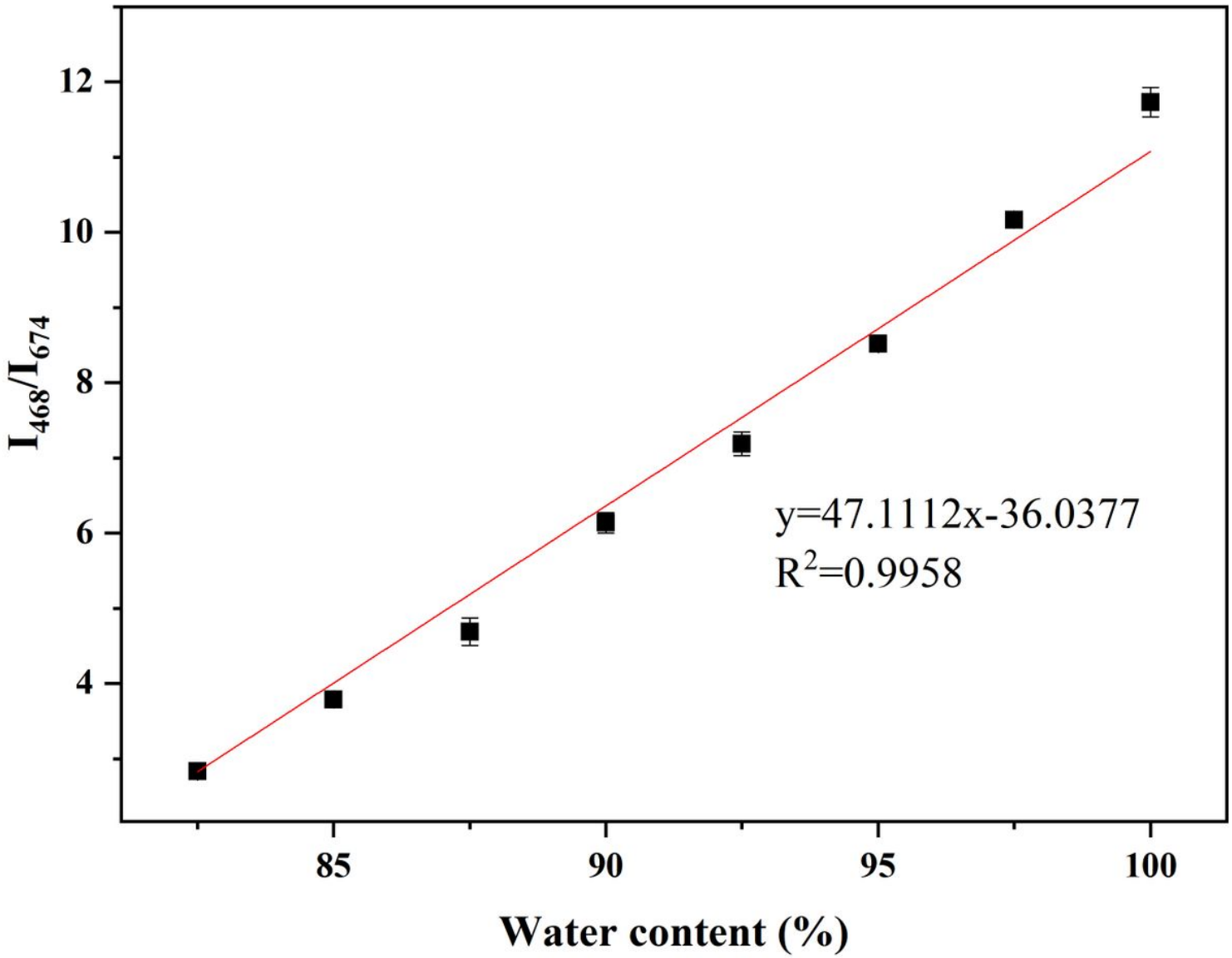

Figure 8

Linear fitting diagram

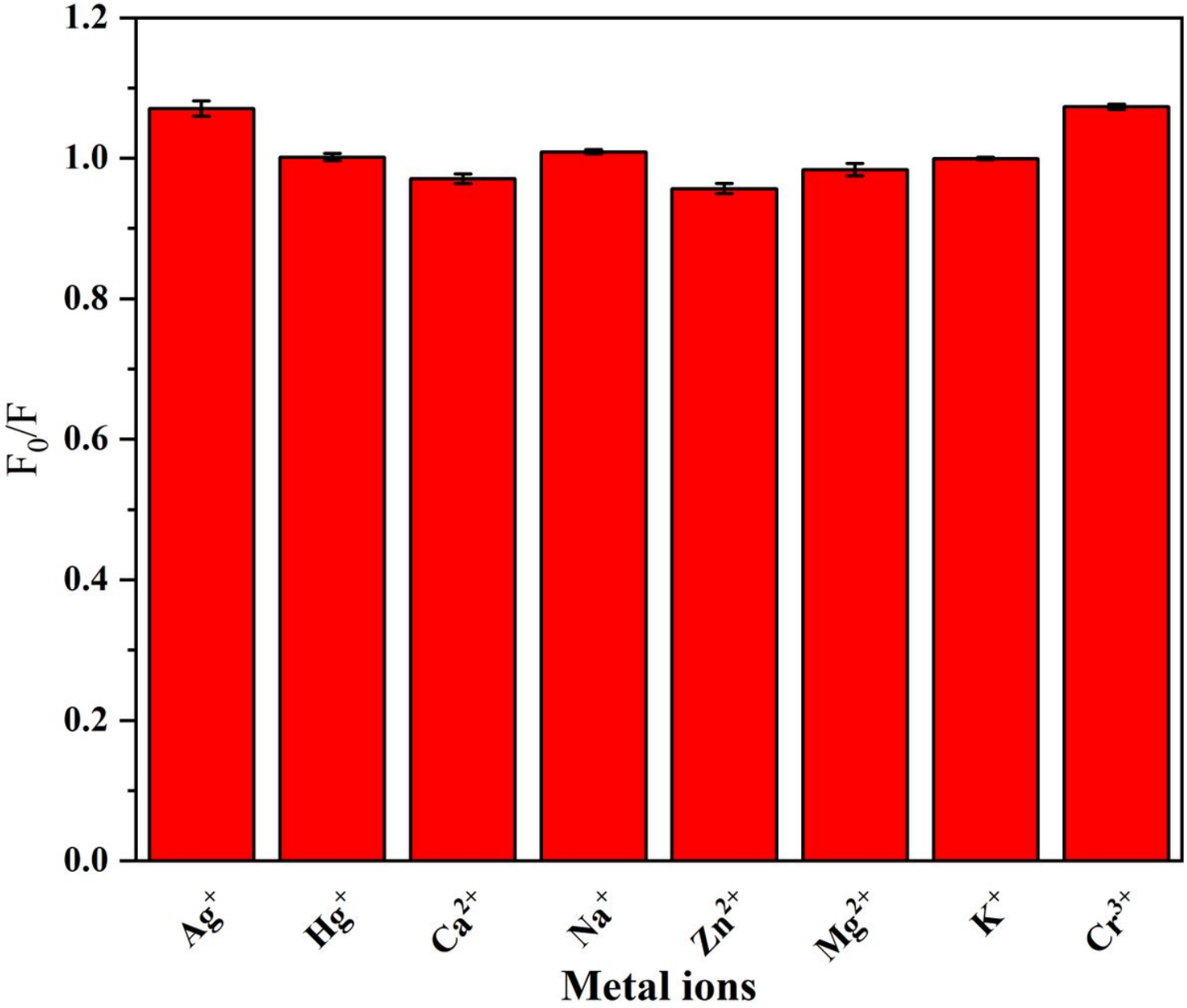

Figure 9

Heavy metal interference