

## Design a $\text{Cu}^{2+}$ Fluorescent Probe Derived from Pyrene

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## Abstract

A  $\text{Cu}^{2+}$  probe based on pyrene derivatives was introduced. Compared to other metal ions, it exhibited a highly selective and sensitive fluorescence response to  $\text{Cu}^{2+}$ .

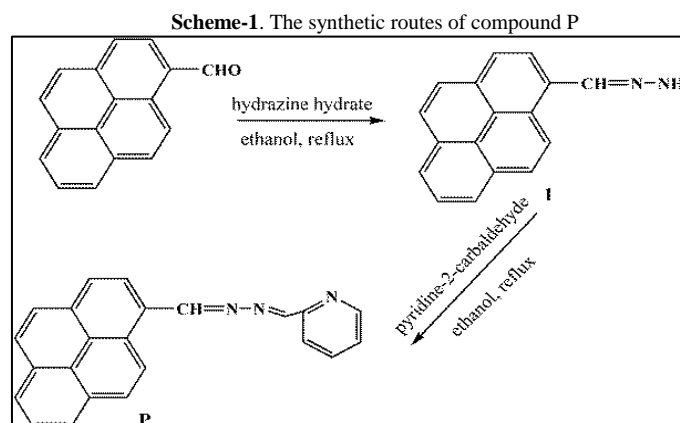
**Keywords:**  $\text{Cu}^{2+}$ ; Fluorescent probe; Pyrene derivatives.

## 1. Introduction

Copper (Cu) is an important trace element and nutrient element required by the human body. It plays a very important role to maintain the body's activities.  $\text{Cu}^{2+}$  deficiency will make the body's metabolism disorder, resulting in anemia, visual impairment, poor hair quality [1-3], etc. Excessive intake of  $\text{Cu}^{2+}$  will also cause a lot of harm, such as children's hyperactivity, middle-aged and elderly arthritis, diabetes, bleeding and kidney disorders, [4-8]. So effective detection of  $\text{Cu}^{2+}$  concentration in the environment is an important way to prevent  $\text{Cu}^{2+}$  from harming the body.

Among the many detection methods, fluorescence analysis has the advantages of high sensitivity, simple operation, low cost, strong specificity, wide use, et al, so it has attracted much attention, and now this method has been widely used in various detections [9-13]. The molecular structure of pyrene is composed of four benzene rings, so there is a large conjugated system [14-16]. The pyrene molecule is a good rigid plane and a phosphor with high fluorescence efficiency, strong absorption of light, good optical stability and a long fluorescence lifetime<sup>1</sup>.

In our study, pyreneformaldehyde and hydrazine hydrate were used to synthesize intermediate compound 1, and compound 1 was combined with pyridine-2-carbaldehyde to generate target probe P. In this work, it was proposed to graft the fluorescent group derived from pyrene derivative with amino group derived from pyridine derivative. A new  $\text{Cu}^{2+}$  fluorescent probe with Schiff base structure to achieve efficient detection of  $\text{Cu}^{2+}$  was designed and synthesized. The synthesis route of the probe was shown in Scheme 1.



## 2. Experimental Section

### 2.1. Reagents and Instruments

All reagents are commercially available analytical pure grades. Pyrene formaldehyde; Pyridine-2-carbaldehyde; EDTA; HEPES; Dimethyl sulfoxide; Absolute ethanol; 37% concentrated hydrochloric acid; Sodium hydroxide .

U-2910 spectrophotometer and F-4600 fluorescence spectrometer were used

### 2.2. Synthesis of P

Synthesis of compound 1: 0.2 g (0.87 mmol) pyrene formaldehyde was added to a round bottom flask, and then 7.8 mL of 85% hydrazine hydrate and 50 mL absolute ethanol were added, which were heated and refluxed for 4 h, the yellow solid was generated by suction after cooling to room temperature.

Synthesis of probe P: 50 mg compound 1 (0.17 mmol) and 25  $\mu$ L of pyridine-2-carbaldehyde (2.6 mmol) were added to the round bottom flask and an appropriate amount of ethanol was also added. After the reaction was heated and refluxed for 4 h, orange solid was filtered with a yield of 71.7%.

### 2.3. General Spectroscopic Methods

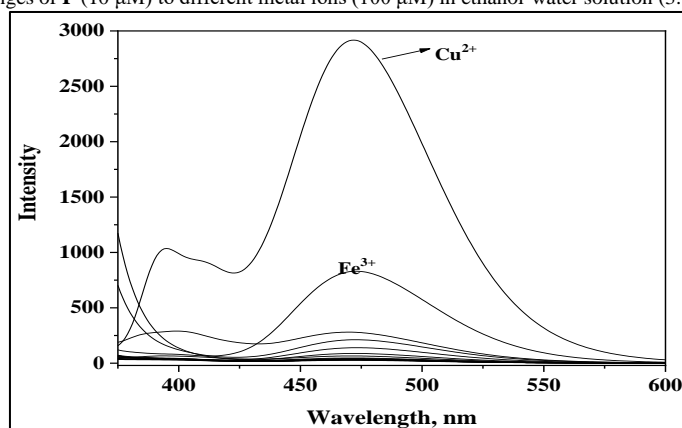
All fluorescence spectra were recorded at room temperature (25  $^{\circ}$ C). The probe P stock concentration was 1 mM. The excitation wavelength was 340 nm and the excitation and emission slits were both of 10 nm.

## 3. Results and Discussion

### 3.1. Fluorescence Selectivity of P

The selective identification of major metal ions by probe P was shown in Fig. 1. It can be seen that with the addition of  $\text{Cu}^{2+}$ , bimodal emission at 401 nm and 472 nm appeared which was attributed to the characteristic emission peak of pyrene, and the fluorescence intensity was significantly enhanced, while other metal ions did not caused remarkable change of fluorescence under the same experimental conditions. The experimental results indicated that probe P had specific recognition for  $\text{Cu}^{2+}$ .

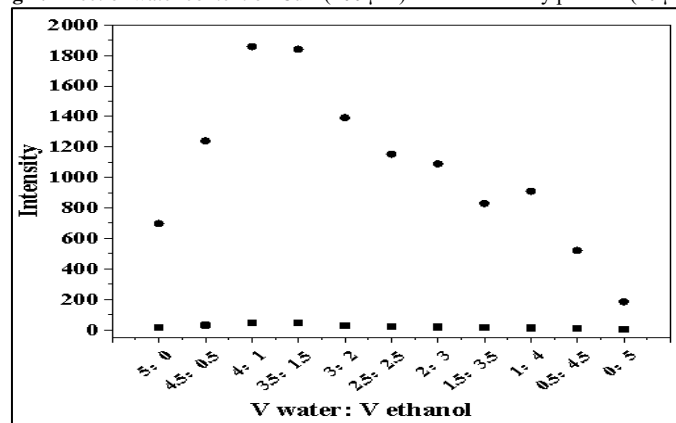
Fig-1. Fluorescent emission changes of P (10  $\mu$ M) to different metal ions (100  $\mu$ M) in ethanol-water solution (3:7, V:V, pH 7.5, 20 mM HEPES)



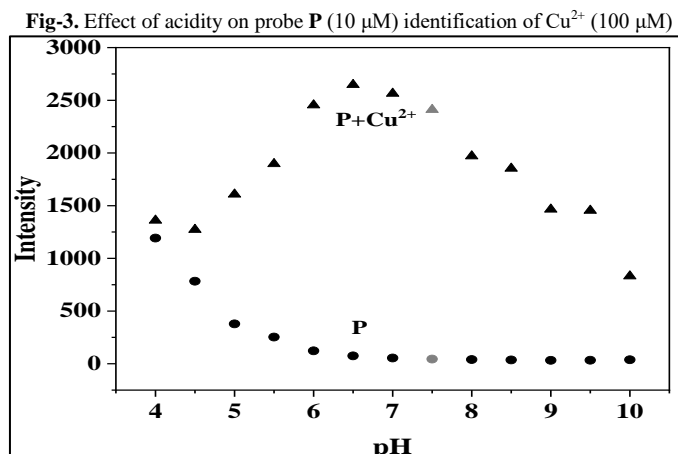
### 3.2. Experimental Condition of P

In order to investigate the influence of water on the performance of probe P, as shown in Fig. 2. As the content of ethanol in media continued to increase, a new fluorescence peak at 472 nm appeared and the fluorescence intensity increased and then decreased, when V water: V ethanol = 7:3, the maximum fluorescence intensity at 472 nm was reached, Therefore, V water: V ethanol = 7:3 was chosen for the subsequent experiment, which can be seen that the water solubility of probe P was very advantageous in detecting  $\text{Cu}^{2+}$  in water.

Fig-2. Effect of water content on  $\text{Cu}^{2+}$  (100  $\mu$ M) identification by probe P (10  $\mu$ M)



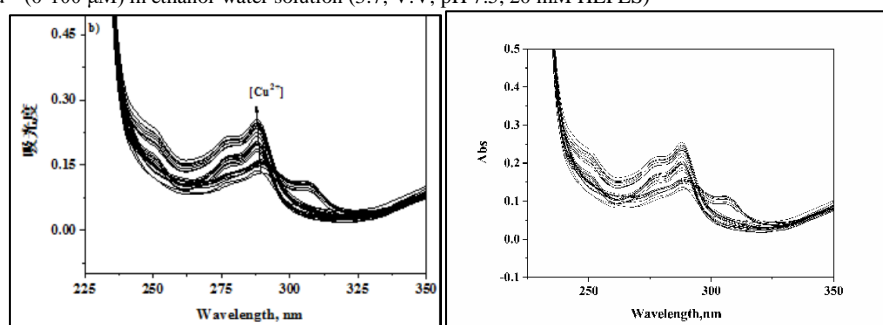
The influence of the acidity was also investigated. As shown in Fig. 3, in the pH 4-7.5 range, the fluorescence intensity at 472 nm had a significant enhancement, and the fluorescence intensity at 472 nm in the pH 8-10 range had a significant downward trend, possibly because  $\text{Cu}^{2+}$  would precipitate in an alkaline environment. In order to better play the performance of the probe, other performance studies were carried out at pH 7.5. The fluorescence intensity in a large pH span was obvious and stable, indicating that it had strong adaptability to the environment.



### 3.3. Fluorescence Sensitivity of P

The effect of  $\text{Cu}^{2+}$  concentration on fluorescence spectra and UV-vis spectra was further investigated, as shown in Fig. 4. The experimental results showed that with the increase of  $\text{Cu}^{2+}$  concentration, the characteristic fluorescence peak intensity of pyrene derivatives at 472 nm also enhanced, and the fluorescence intensity at 472 nm had a good linear relationship toward  $\text{Cu}^{2+}$  (1-10  $\mu\text{M}$ ), and the detection limit was 0.33  $\mu\text{M}$  based on  $3\sigma$  rule. Moreover, the increase of  $\text{Cu}^{2+}$  concentration caused the absorbance at 285 nm to increase regularly, which was attributed to the characteristic absorption peak of pyrene. This experiment strongly proved that probe P was an ideal probe for  $\text{Cu}^{2+}$  detection.

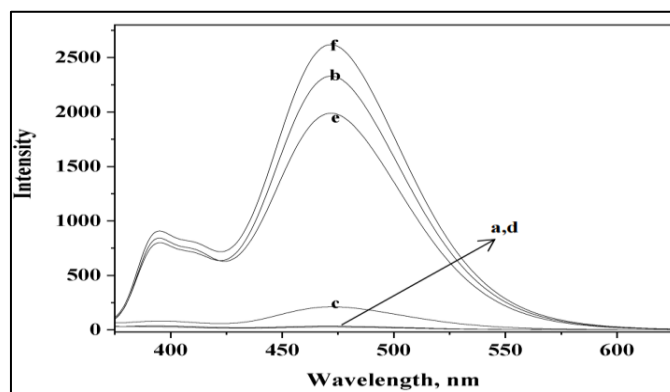
**Fig-4a.)** Fluorescence titration plot of  $\text{Cu}^{2+}$  (0-100  $\mu\text{M}$ ) on probe P (10  $\mu\text{M}$ ); b) UV-Vis absorption titration plot of probe P (10  $\mu\text{M}$ ) at different concentrations of  $\text{Cu}^{2+}$  (0-100  $\mu\text{M}$ ) in ethanol-water solution (3:7, V:V, pH 7.5, 20 mM HEPES)



### 3.4. Reversibility of P

Whether the reversibility of the probe was directly related to the reuse of P, as shown in Fig. 5. After the addition of  $\text{Cu}^{2+}$ , due to the selectivity of the probe for  $\text{Cu}^{2+}$ , the probe had a fluorescence peak at 472 nm (Fig. 5b), when an equal amount of EDTA was added, the intensity fell off, because  $\text{Cu}^{2+}$  reacted with EDTA to form a complex to weak the fluorescence intensity at 472 nm (Fig. 5c-d), and when excess  $\text{Cu}^{2+}$  was added again, the fluorescence intensity at 472 nm recovered again, and the fluorescence intensity became stronger when more  $\text{Cu}^{2+}$  was added (Fig. 5e-f), and the experiment proved that the probe P had certain reusability.

**Fig-5.** Reversibility of the P- $\text{Cu}^{2+}$  system in ethanol-water solution (3:7, V:V, pH 7.5, 20 mM HEPES). a) P (10  $\mu\text{M}$ ); b) P (10  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (10  $\mu\text{M}$ ); c) P (10  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (10  $\mu\text{M}$ ) + EDTA (10  $\mu\text{M}$ ); d) P (10  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (10  $\mu\text{M}$ ) + EDTA (50  $\mu\text{M}$ ); e) P (10  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (10  $\mu\text{M}$ ) + EDTA (50  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (50  $\mu\text{M}$ ); f) P (10  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (10  $\mu\text{M}$ ) + EDTA (50  $\mu\text{M}$ ) +  $\text{Cu}^{2+}$  (100  $\mu\text{M}$ ).



## 4. Conclusions

In this paper, the design of pyrene derivatives was carried out to form a molecular fluorescent probe containing pyridine-containing heterocycles, and the combination of the probe with  $\text{Cu}^{2+}$  caused the conjugation system of pyrene derivatives to increase fluorescence enhancement, and the  $\text{Cu}^{2+}$  fluorescent probe based on pyrene displayed important practical significance for the detection of  $\text{Cu}^{2+}$  in water and the development of related fluorescent probes.

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