

# Characterization of A Hg<sup>2+</sup>-Selective Fluorescent Probe Based on Rhodamine B Derivative

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**Abstract** - A Hg<sup>2+</sup>-selective fluorescent probe derived from Rhodamine B was synthesized and characterized. At 589 nm, the fluorescence intensity of the probe was enhanced with the addition of Hg<sup>2+</sup>. In the range of 0.5-7.0 μM, there is an obvious linear relationship between the fluorescent intensity and the concentration of Hg<sup>2+</sup>, and the detection limit was 0.17 μM. The UV-vis spectra of the probe also indicate that the probe has good selectivity and sensitivity to Hg<sup>2+</sup>.

**Keywords** — Fluorescent probe; Rhodamine B; Hg<sup>2+</sup>; Heavy metals; Detection methods

## I. INTRODUCTION

The detection of environment contamination caused by heavy metals has become an interesting study fields. Mercury can exist as elemental, inorganic and organic forms in environment and Hg<sup>2+</sup> is a carcinogenic and caustic material with high biological toxicity [1-3]. Therefore, it is of great importance to develop efficient analytical methods to detect Hg<sup>2+</sup> in environment and biosystems. During the past few years, different analytical methods including inductively coupled plasma-mass spectrometry [4], electrochemical methods[5-6] and UV-vis spectrometry [7] have been designed for the detection of Hg<sup>2+</sup>, but most of them are complicated, costly, especially not suitable for in vitro/vivo application. Recently, the design of selective and sensitive fluorescent probes has attracted considerable interest due to its remarkable advantage such as low cost, operational simplicity, nondestructiveness, et al [8-11]. Variety of fluorescent probes with high performance for detection of environmental and/or biological relative targets have been proposed [12-14]. Compared with some “turn-on” fluorescent probes for metal ions, such as Cu<sup>2+</sup> [15-17], Al<sup>3+</sup> [18-20] and Mg<sup>2+</sup> [20,21], which have been successfully designed, Hg<sup>2+</sup>-selective probes are few [22-24]. Therefore, the synthesis of fluorescence enhancement type Hg<sup>2+</sup>-selective probes is still a research challenge.

Among the reported fluorescent probes, rhodamine B type ones become the first choice for research because of the fact that the addition of the target substance cause a visible color change of the tested sample [17,19,22]. According to this, a rhodamine based Hg<sup>2+</sup>-selective fluorescent probe P was designed and synthesized using rhodamine B and p-chloronitrobenzene as starting materials.

## II. EXPERIMENTAL SECTION

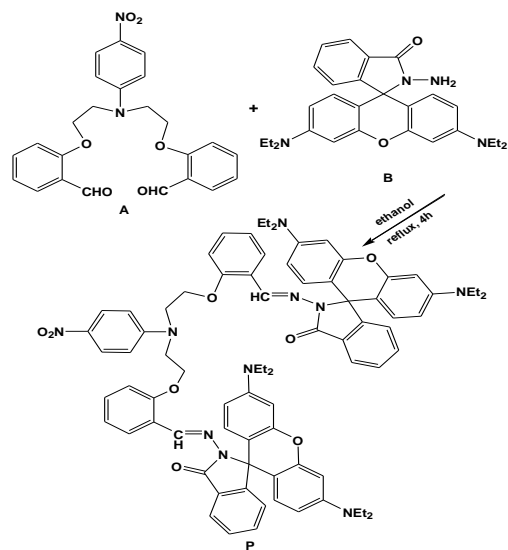
### A. Reagents and Instruments

All reagents are commercially available and used directly. The metal salts used in the experiment were NaCl, KCl, AgNO<sub>3</sub>, CaCl<sub>2</sub>·2H<sub>2</sub>O, MgCl<sub>2</sub>·6H<sub>2</sub>O, MnCl<sub>2</sub>·4H<sub>2</sub>O, HgCl<sub>2</sub>, CuCl<sub>2</sub>·2H<sub>2</sub>O, CdCl<sub>2</sub>, CrCl<sub>3</sub>·6H<sub>2</sub>O, PbCl<sub>2</sub>, FeCl<sub>2</sub>·4H<sub>2</sub>O, Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, CoCl<sub>2</sub>·6H<sub>2</sub>O, and NiCl<sub>2</sub>·6H<sub>2</sub>O.

UV-vis spectra were carried on a Hitachi U-2910 spectrophotometric. Fluorescent spectra were recorded using a Hitachi F-4600 spectrofluorometer. Nuclear magnetic resonance (NMR) spectra were measured with a Bruker AV 400 instrument and chemical shifts are given in ppm from tetramethylsilane (TMS).

### B. Synthesis of P

Synthesis route of P was shown in Scheme 1.



**Scheme 1. Synthesis route of P**

Compound A and B were synthesized according to the reported methods [25,26], respectively.

Compound A (0.15 mmol) and B (0.31 mmol) were reacted in ethanol (40 mL) and stirred under reflux for 4 h, and then the mixture was cooled to room temperature, the precipitate so obtained was filtered and dried in vacuum. The



product was used directly.  $^1\text{H NMR}$ : 9.13 (s, 2H), 8.00 (d, 2H,  $J=9.20$ ), 7.88 (d, 2H,  $J=8.40$ ), 7.75 (t, 4H,  $J=9.20$ ), 7.56 (t, 4H,  $J=7.20$ ), 7.18 (t, 2H,  $J=8.60$ ), 7.05 (d, 2H,  $J=7.60$ ), 6.97 (t, 4H,  $J=6.40$ ), 6.85 (d, 2H,  $J=8.40$ ), 6.81 (d, 2H,  $J=7.60$ ), 6.36 (t, 4H,  $J=6.40$ ), 4.15 (t, 4H), 3.93 (t, 4H), 3.33 (m, 16H,  $J=7.60$ ), 1.07 (t, 24H,  $J=6.80$ ).

### C. General Spectroscopic Methods

The stock solutions of P and metal ions (1.0 mM) were obtained by dissolving salts and P in deionized water and dimethylsulfoxide, respectively, and the testing solutions was freshly prepared before measurements by diluting the stock solutions.

## III. RESULTS AND DISCUSSION

### A. Selectivity Measurement

The selectivity of probe P was firstly studied using fluorescent and UV-vis method. The tested metal ions were  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ag}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Cr}^{3+}$ . Among the tested ions, only the addition of  $\text{Hg}^{2+}$  (10  $\mu\text{M}$ ) caused an obvious fluorescent enhancement at 589 nm in ethanol,  $\text{Co}^{2+}$  and  $\text{Cu}^{2+}$  caused a weak change at 575 nm and didn't affect the detection of  $\text{Hg}^{2+}$  (Fig. 1a). The UV-vis spectra of P (10  $\mu\text{M}$ ) was also investigated in ethanol with the addition of the tested metal ions (10  $\mu\text{M}$ ) (Fig. 1b), and a absorbance at 555 nm was observed. All these results showed that this probe has good selectivity to  $\text{Hg}^{2+}$  compared to other tested metal ions.

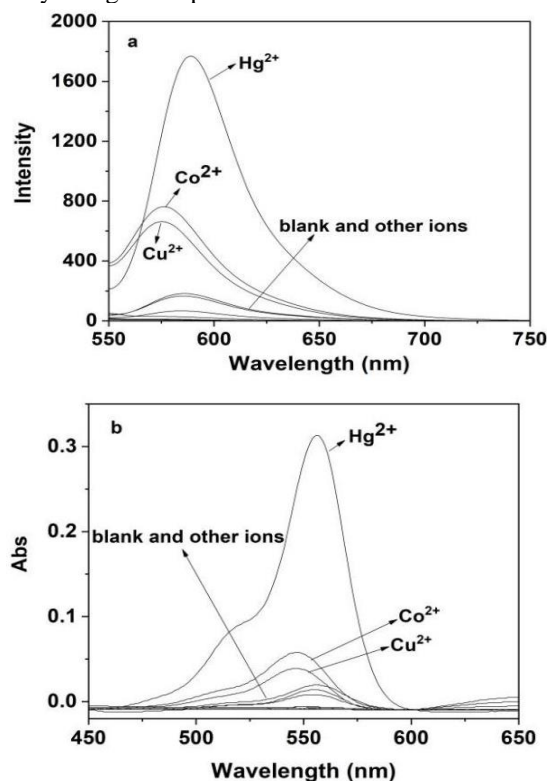


Fig. 1 Fluorescence and UV-vis spectra of P (10  $\mu\text{M}$ ) in ethanol after adding different ions (10  $\mu\text{M}$ )

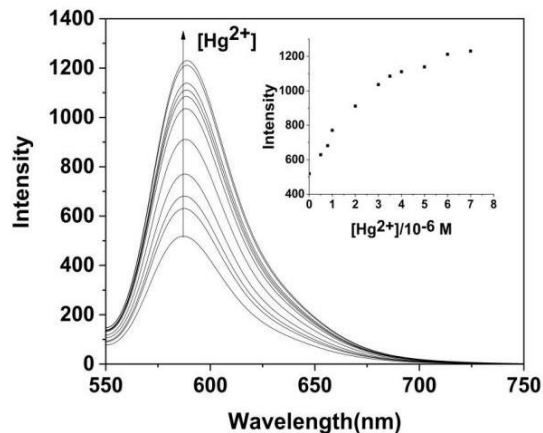


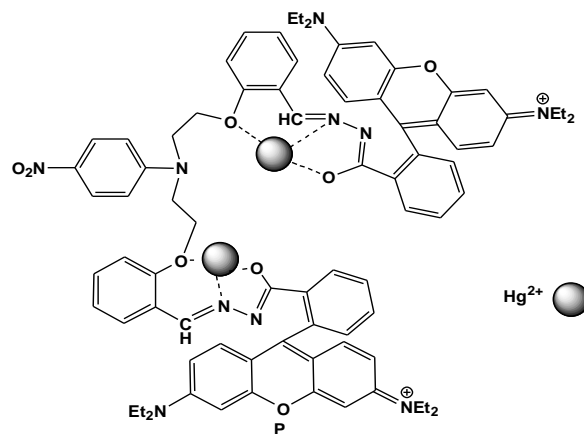
Fig. 2 Fluorescence response of P (10  $\mu\text{M}$ ) with various concentrations of  $\text{Hg}^{2+}$  (0-10.0  $\mu\text{M}$ ) in ethanol. Insert: linear relationship diagram of probe P and  $\text{Hg}^{2+}$  concentration

### B. Fluorescent Titration Experiment of P with $\text{Hg}^{2+}$

Fluorescent titration experiment was carried out for the further study of P with  $\text{Hg}^{2+}$  (Fig. 2). From the results we can see that the fluorescent intensity at 445 nm enhanced with the increase of content of  $\text{Hg}^{2+}$ , and a linear relationship was found in the range of 0.5-7.0  $\mu\text{M}$  with a detection limit of 0.17  $\mu\text{M}$   $\text{Hg}^{2+}$ .

### C. Coordination Mechanism of P with $\text{Hg}^{2+}$

According to the above-mentioned results, the binding mode of P with  $\text{Hg}^{2+}$  was proposed as shown in Scheme 2. The addition of  $\text{Hg}^{2+}$  caused the ring-opening of the corresponding rhodamine spirolactam, which induced an enhancement of the fluorescent intensity.



Scheme 2. Binding mode of P with  $\text{Hg}^{2+}$

## IV. CONCLUSIONS

In summary, a  $\text{Hg}^{2+}$ -selective fluorescent probe was successfully characterized, compared to other tested metal ions, this probe showed good selectivity and sensitivity to

Hg<sup>2+</sup>. We believe that this study will promote the development of the design of ligands for the detection of Hg<sup>2+</sup>.

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