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Original Article

Construction of a Hg^{2+}-Sensitive Fluorescent Probe Derived from Rhodamine B

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Abstract - *A rhodamine B-based Hg^{2+}-sensitive fluorescent probe P was characterized in H_2O-EtOH solution (7:3, v:v, pH 7.0, 2 mM HEPES). Over other tested cations, only the addition of Hg^{2+} induced the opening of the spiral ring structure of P, and a fluorescence enhancement occurred at 595 nm with a naked eye observed color change from colorless to pink. Using Na_2S as a complexing agent, the reversibility of the P-Hg^{2+} system was proved. The linear range of P for Hg^{2+} was 9.0×10^{-6}-3.0×10^{-5} M with a detection limit of 3.0×10^{-6} M.*

Keywords - *Rhodamine B, fluorescent probe, Hg^{2+}, off-on, fluorescent enhancement.*

1. Introduction

The accumulation property of ecosystems makes Hg^{2+} a toxic heavy metal, with its detrimental effects on the nervous system having been extensively studied [1,2]. Due to the lipophilic nature of methylmercury, it can bioaccumulate along the food chain, significantly elevating health risks [3]. Therefore, monitoring and controlling Hg^{2+} concentrations in vitro and in vivo is crucial for environmental and human health.

Fluorescent probe detection methods offer numerous advantages, including good selectivity, high sensitivity, and ease of use [4,5]. Many probes with excellent responses to target ions have been reported.

Rhodamine derivatives, first utilized for detecting Cu^{2+} ions in 1997 [6], have garnered significant interest due to their outstanding optical properties.

Studies have shown that by altering the pH of the buffer and modifying the rhodamine structure, specific responses to different targets can be achieved, thereby expanding the applicability of these derivatives. 2006, a Hg^{2+}-selective rhodamine derivative was synthesized and characterized [7]. Since then, numerous fluorescent probes based on rhodamine derivatives for detecting Hg^{2+} ions have been developed and reported [8-10].

Considering this, a rhodamine B derivative labelled P was synthesized and characterized as a selective fluorescent probe for Hg^{2+} (Fig. 1).

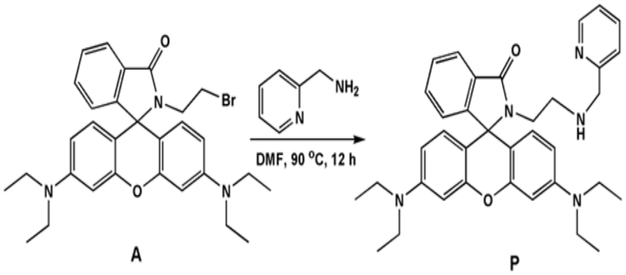

Fig. 1 Synthesis route of probe P.

2. Experimental Section

2.1. Reagents and Instruments

All reagents used were of analytical grade and did not require further purification prior to use. Fluorescent data were collected using a Hitachi 4600 spectrofluorometer. Mass spectrometry analyses were performed on a Thermo TSQ Quantum Access system coupled with an Agilent 1100.

2.2. Synthesis of P

Compound A was synthesized according to the reported method.

Synthesis of P: 1 mmol of compound A and 1.2 mmol of 2-(Aminomethyl)pyridine was added to a three-necked flask containing 30 mL of DMF. The reaction was conducted at 90 °C for 12 hours and then cooled to room temperature. The solution was evaporated under vacuum, and the solid product was purified by column chromatography using a solvent system of 1:1 (v:v) $C_4H_8O_2$: CH_2Cl_2, yielding a yellow powdery solid P. The yield was 70.4%. MS (ESI) m/z: 598.51 $[M-H^+]^+$.

2.3. Basic Spectroscopic Procedures

Stock solutions of cations at a concentration of 1.0 mM were prepared by dissolving the corresponding salts in deionized H_2O. Compound P was dissolved in DMSO. The slit widths were set to 10 nm and 20 nm, respectively, and the excitation wavelength was set to 520 nm.

3. Results and Discussion

3.1. Solvent and pH effect on the Response of P

Excess H_2O can lead to the precipitation of the probe; however, it is essential to detect H_2O samples for the practical application of the probe. Therefore, the ratio of H_2O to EtOH in the test item must be regulated. Firstly, the containing of H_2O on the fluorescent response of P was studied (Fig. 2). With the increasing of H_2O content (v:v), the fluorescent intensity arrived at maximum at the ratio of 7:3 (H_2O: EtOH, v:v). Consequently, the H_2O-EtOH solution in this ratio was used for the subsequent experiments. Next, the effect of pH was examined by adjusting the P and P+Hg^{2+} systems using 1.0 M HCl or NaOH (Fig. 3). Based on these results, pH 7.0 was selected as the testing pH to align with the pH of real environmental samples. Thus, the detection medium was established as an H_2O-EtOH solution (7:3, v:v) at pH 7.0, supplemented with 2 mM HEPES.

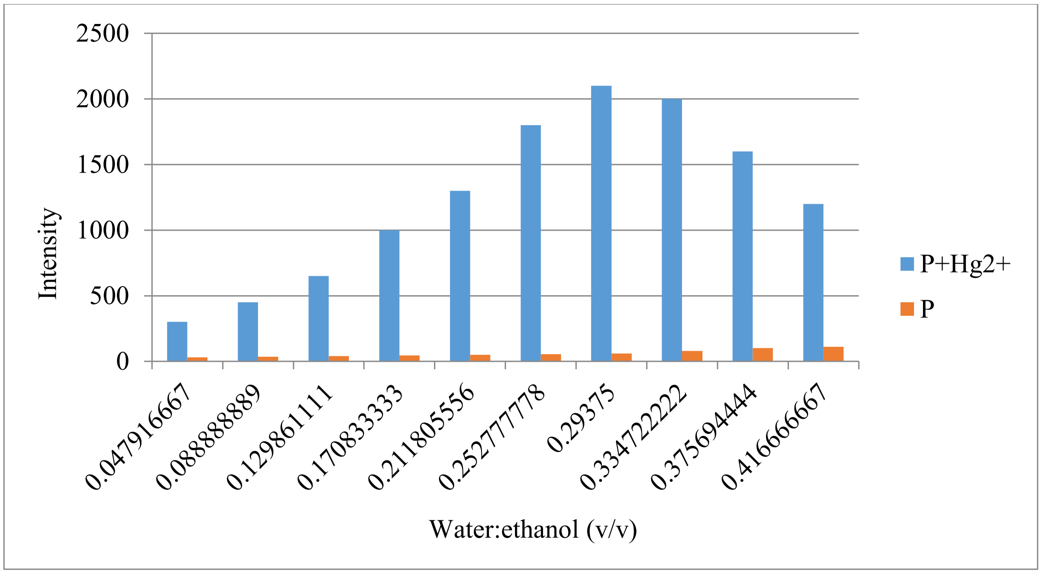

Fig. 2 Influences of H_2O: EtOH (v:v) on the fluorescence response of P and P (10 µM) plus Hg^{2+}

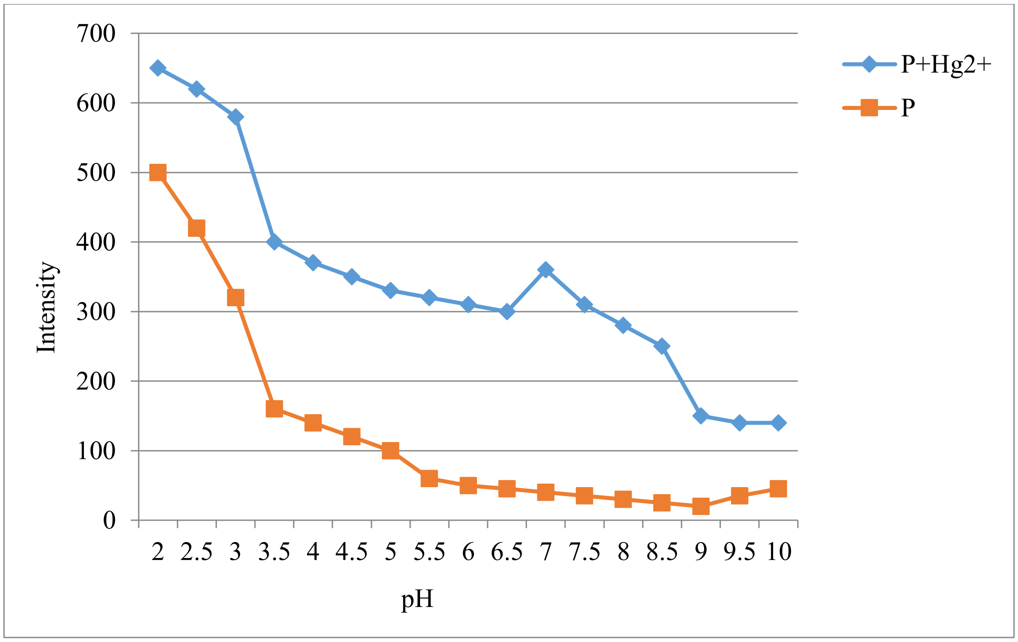

Fig. 3 pH effect on the fluorescent response of P (10 µM) (●) and P (10 µM) plus Hg^{2+} (100 µM) (■).

3.2. Selectivity measurement of P

Selectivity is a crucial property of probes, as it determines their suitability for testing complex samples. In a H_2O-EtOH solution (7:3, v:v, pH 7.0, 2 mM HEPES), the selectivity of P (10 μM) was evaluated (Fig. 4). The testing ions included Ag^+, Al^{3+}, Na^+, K^+, Fe^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+} (all at 100 μM). As shown in Fig. 4, only the addition of Hg^{2+} resulted in a significant fluorescent enhancement at 593 nm, while the other metal ions quenched the fluorescence of P. This indicates that P is a "turn-on" type fluorescent probe selectively for Hg^{2+}.

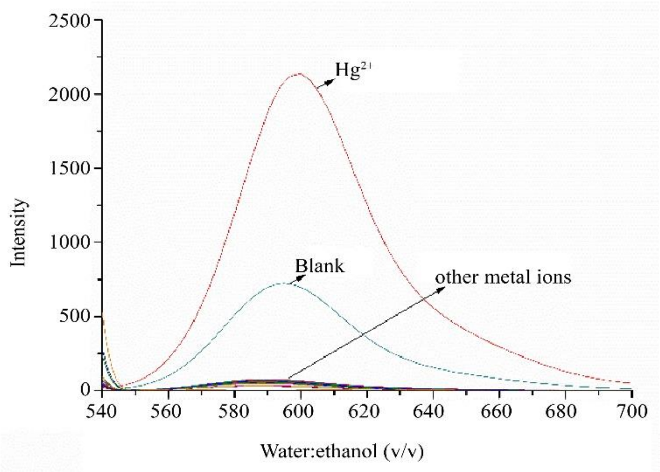

Fig. 4 Fluorescent response of P (10 μM) to the testing cations (100 μM) in H_2O-EtOH solution (7:3, v:v, pH 7.0, 2 mM HEPES).

3.3. Sensitivity study of P

To evaluate the sensitivity of probe P, a fluorescent titration experiment was conducted in an H_2O-EtOH solution (7:3, v:v, pH 7.0, 2 mM HEPES) (Fig. 5). Upon the addition of Hg^{2+} (0-100 μM) to the P solution, the fluorescence intensity gradually increased, demonstrating a linear response of P to the concentration of Hg^{2+} in the range of 9.0×10^{-6} M to 3.0×10^{-5} M. The detection limit was calculated to be 3.0×10^{-6} M for Hg^{2+}.

3.4. Recognition mechanism of P with Hg^{2+}

The binding mode and recognition mechanism were inferred from the experimental results, as illustrated in Fig. 6. The addition of Hg^{2+} induced the opening of the spiral ring structure of probe P, resulting in the "off-on" fluorescence response associated with Hg^{2+} binding and subsequent fluorescent enhancement.

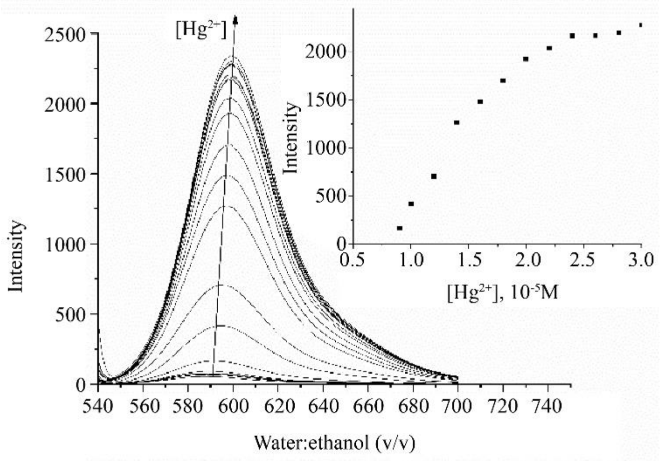

Fig. 5 Fluorescent titration experiment of P (10 μM) to Hg^{2+} (0-100 μM) in H_2O-EtOH solution (7:3, v:v, pH 7.0, 2 mM HEPES)

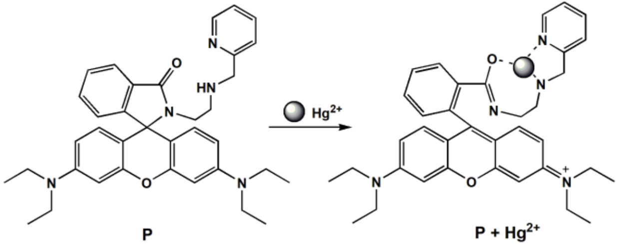

Fig. 6 Proposed binding mode of P-Hg^{2+}.

4. Conclusion

A Rhodamine B-based derivative was characterized as an Hg^{2+} sensitive fluorescent probe with good selectivity, and a "turn-on" type of fluorescent response was observed with the naked eye.

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