

Characterization of Zn²⁺ Selective Fluorescence Probe Based on Pyrene Derivatives

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Abstract

A Zn²⁺-selective fluorescent probe derived from pyrene derivatives was synthesized and characterized based on C=N isomerization which indicated that the O and N atoms in P indeed played important roles in the course of binding with Zn²⁺. The fluorescence intensity of the probe at 522 nm was enhanced with the addition of Zn²⁺ over other metal ions. In the concentration range of 1-9 μM, there was an obvious linear correlation between the fluorescent intensity at 522 nm and the concentration of Zn²⁺. The limit of detection (LOD) was obtained as low as 0.33 μM of Zn²⁺. The UV-vis spectra also indicated that the binding of between the probe and Zn²⁺. The design concept will provide ideas for the development of fluorescent probes.

Keywords: Fluorescent probe; Pyrene derivatives; Zn²⁺; Heavy metals.

1. Introduction

Metal ions, as indispensable substances in human survival and development and life activities, are essential trace elements for human body. When excessive metal ions enter human body through respiration, diet and other ways, they will cause harm to human body. Meanwhile, their enrichment in the environment will also cause environmental pollution and ecological damage. There is a need for efficient and sensitive methods to detect metal ions in the environment and organisms that are easy to operate and provide fast and accurate results. Among the current scientific detection methods for metal ions [1-8], most of them rely on large instruments. Fluorescent probe has gained a high popularity in the detection of metal ions in the environment and ecology due to its excellent characteristics such as wide source, easy modification, high sensitivity, high selectivity, convenient operation and rapid response. The development and exploration of fluorescence probes with better performance has gradually become a research hotspot, so it is of great significance to design and synthesize a fluorescence probe with high selectivity and high sensitivity in aqueous solution. Zn²⁺ deficiency can lead to developmental delays and neurological diseases. However, excess will cause a series of physiological dysfunction in human immunity [9]. Due to the 3d¹⁰4s⁰ electronic structure of Zn²⁺, there is no vacant d orbital, so it does not have the D-D electronic transition that transition metal elements usually have. At the same time, because Zn²⁺ has no unpaired single electron, it does not exhibit any magnetism, so that it has no detectable spectral and nuclear magnetic signal, resulting in certain difficulties in the determination of Zn²⁺. Reported fluorescent probes widely use signaling mechanisms of photoinduced electron/energy transfer (PET) [10], excimer/exciple formation [11], intramolecular charge transfer (ICT) [12], Fluorescence Resonance Energy Transfer (FRET) [13] C=N isomerization has been applied to design fluorescent probes for the detection of various metal ions. It was found that C=N isomerization is the predominant decay process of excited states in compounds with unbridged C=N structure, which usually results in nonfluorescence of those compounds. In contrast, it is reasonable to amplify fluorescence upon binding with metal ions to an elaborately designed molecule framework by blocking C=N isomerization rather than by covalent bridging of the C=N bond [14]. With this in mind, we can reasonably expect that C=N isomerization may also be inhibited by complexation to the recognition moiety linked to the fluorophore. Pyrene and its derivatives can form excimer, and the fluorescence quantum produced by the excited state has high yield and long time, the fluorescence emission

spectrum is different from that of monomer, and the changes in the microenvironment are extremely sensitive, etc., so that this kind of derivative is widely used in the field of microenvironment changes [15, 16]. Wu et al. synthesized a novel fluorescence “on” probe by pyrene 1-formaldehyde and carbamide hydrazine [17], which can be used to detect Cu^{2+} ions in living cells. Yaru et al. successfully synthesized a fluorescence enhanced Fe^{3+} pyrene probe for the detection of Fe^{3+} [18].

In this paper, pyrene derivatives with better fluorescence properties were selected to design a new probe, which can detect Zn^{2+} qualitatively and quantitatively, respectively.

2. Experimental Section

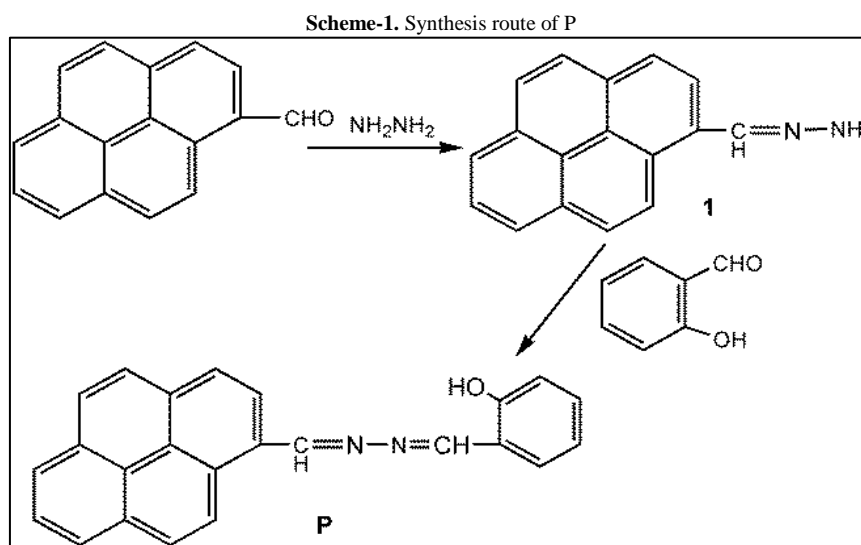
2.1. Reagents and Instruments

Anhydrous ethanol, pyrene formaldehyde, 98% salicylaldehyde, 85% hydrazine hydrate dimethyl sulfoxide, disodium ethylenediaminetetraacetate (EDTA), 4-hydroxyethyl piperazine ethanesulfonic acid (HEPES), Ethyl acetate. Before using the reagents, no special treatment was performed. All reagents were analytical pure.

UV-vis spectra were carried on a Hitachi U-2910 spectrophotometric. Fluorescent spectra were recorded using a Hitachi F-4600 spectrofluorometer.

2.2. Synthesis of P

Synthesis route of P was shown in Scheme 1.



Synthesis of compound 1: In a 250 mL round bottom flask, 0.40 g of pyrene formaldehyde and 16 mL of hydrazine hydrate, an appropriate amount of 50 mL anhydrous ethanol were added. The reaction was heated and refluxed for 4 h, and then cooled to room temperature. The yellow solid product obtained by suction filtration and stored from light.

Synthesis of compound P: 80 mg of compound 1, 145 μL salicylaldehyde (slightly excessive) and an appropriate amount of 50 mL anhydrous ethanol were added into a round bottomed flask. The reaction was heated and refluxed for 6 h, and then cooled to room temperature. Yellow solid P obtained by suction filtration and stored from light.

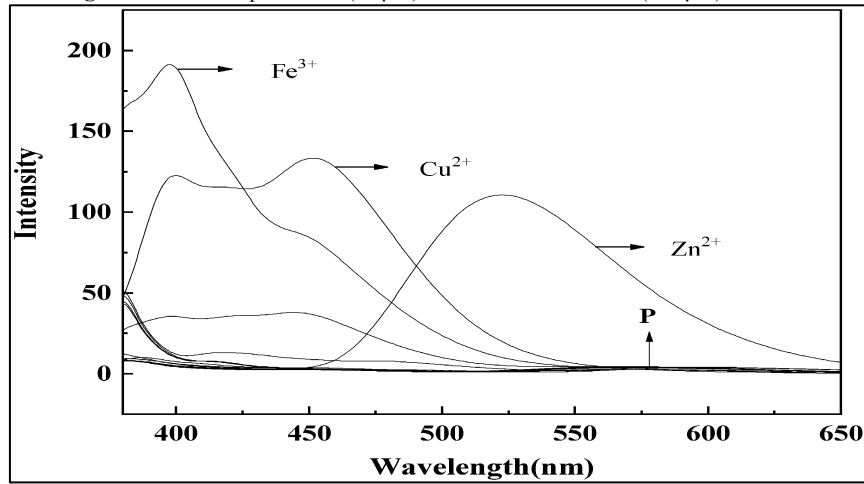
2.3. General Spectroscopic Methods

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

3. Results and Discussion

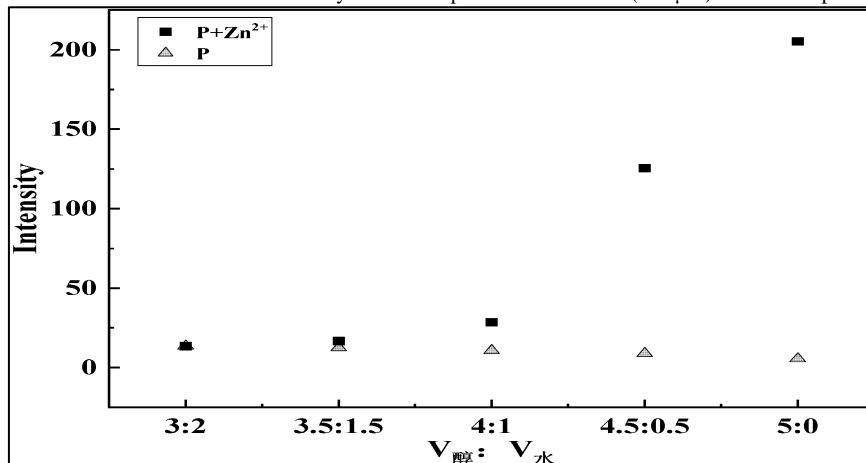
3.1. Selectivity Measurement

To further investigate the selectivity of probe P, fluorescence and UV-vis responses of P toward various metal ions and anions studies were carried out. In the ethanol media, most metal ions (Na^+ , Ag^+ , Zn^{2+} , Pb^{2+} , Cu^{2+} , Co^{2+} , Cd^{2+} , Ni^{2+} , Ca^{2+} , Mg^{2+} , Hg^{2+} , Cr^{3+} and Fe^{3+}). Except for the obvious spectral effects of Fe^{3+} and Cu^{2+} on the probe, however, there was no spectral interference to the determination of Zn^{2+} (Fig. 1). After the addition of Zn^{2+} , the fluorescence peak showed an obvious redshift with peak appeared at 522 nm, which can be used as an excellent probe for the determination of Zn^{2+} .

Fig-1. Fluorescence spectra of P (10 μM) with different metal ions (100 μM) in ethanol

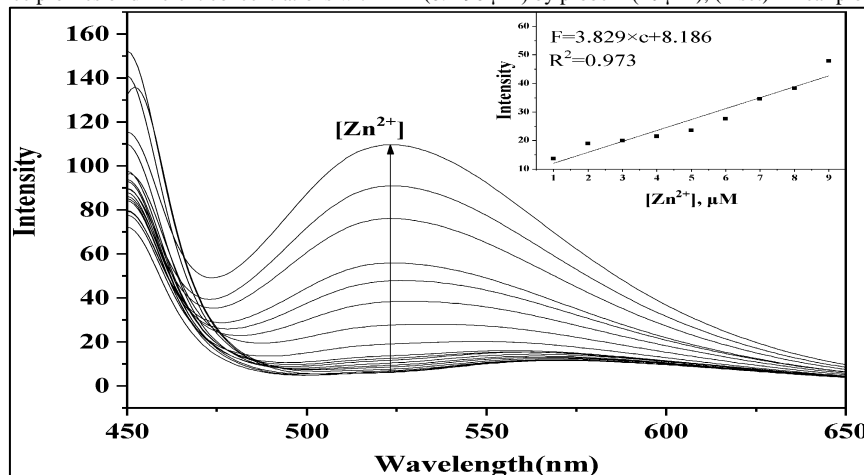
3.2. Effect of Water Content on P Performance

The influence of medium conditions on the ability of P to recognize Zn^{2+} was also examined. It can be seen from Fig. 2 that with the increase of alcohol, the fluorescence intensity of P at 522 nm did not change significantly. However, when Zn^{2+} was added to the solution, the solution had an obvious peak value at 522 nm. With the increase of water content, it can be observed the precipitates, which affected the junction of probe P and Zn^{2+} . With the increase of alcohol content, the fluorescence intensity increased, and subsequent experiments were conducted in the ethanol.

Fig-2. Effect of water content in ethanol-water system on the performance of Zn^{2+} (100 μM) selected for probe P (10 μM)

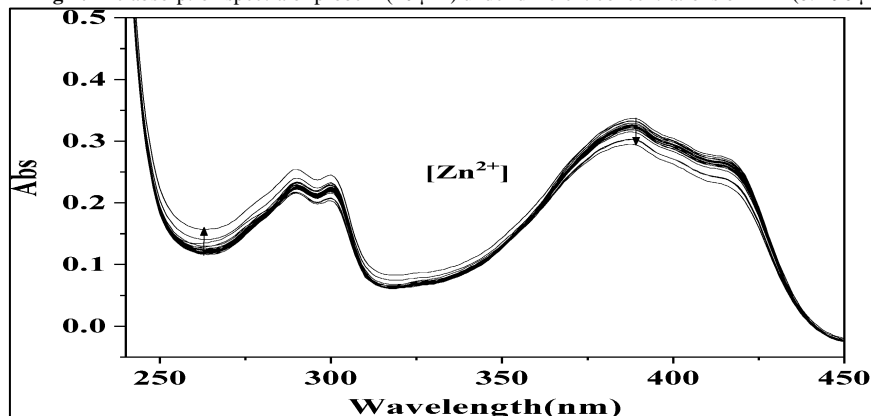
3.3. Titration Experiment of P with Zn^{2+}

As shown in Fig. 3, with the increasing concentration of Zn^{2+} , the fluorescence intensity of the characteristic absorption peak of probe P at 522 nm also gradually increased, and there was a linear relationship in the range of 1-9 μM . This experiment again significantly proves that probe P was an ideal probe for Zn^{2+} . The correlation coefficient was $R^2=0.973$, the linear equation $F=3.829c+8.186$, and the lowest detection limit was 0.33 μM .

Fig-3. Fluorescence profiles of different concentrations with Zn^{2+} (0.1-90 μM) by probe P (10 μM); (Inset) Linear plot of Zn^{2+} (1-9 μM)

With the increase of Zn^{2+} , the absorption spectrum of the system also presented regular changes as displayed in Fig. 4. An isoelectric point appeared at 307 nm, meanwhile, absorbance at 375-450 nm decreased with the increase of concentration with increasing at 250-300 nm, which strongly proved the binding of the probe and Zn^{2+} .

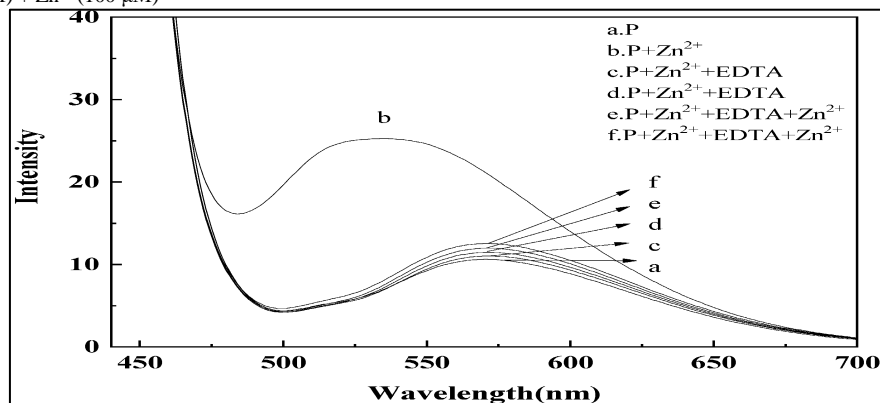
Fig-4. The absorption spectra of probe P (10 μ M) under different concentrations of Zn^{2+} (0.1-90 μ M)



3.4. The Reversibility of P- Zn^{2+}

As shown in the Fig. 5, when Zn^{2+} was added to the probe solution, the fluorescence peak appeared at 522 nm (Fig. 5b), and there was a redshift compared with only 10 μ M probe solution (Fig. 5a). When EDTA was added, the fluorescence intensity at 522 nm decreased sharply, and was almost the same as that of the probe (Fig. 5c). Upon addition of the different concentration of Zn^{2+} , fluorescence intensity at 522 nm did not recover or increase. The possible reason was that EDTA displayed significantly stronger complexation ability with Zn^{2+} . The results showed that the system had poor reversibility.

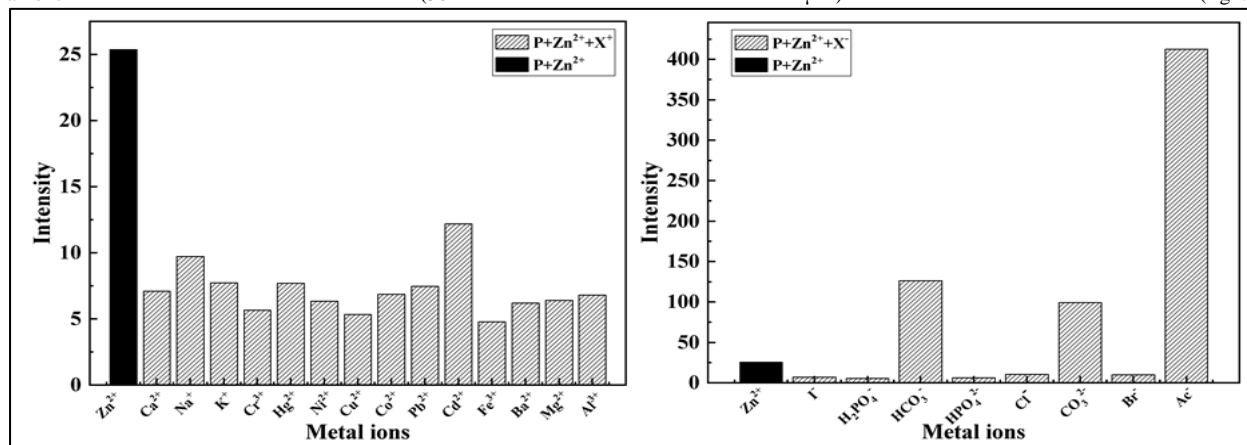
Fig-5. Reversibility of P binding to Zn^{2+} in ethanol : a. P (10 μ M) ; b. P (10 μ M) + Zn^{2+} (10 μ M) ; c. P (10 μ M) + Zn^{2+} (10 μ M) + EDTA (10 μ M); d. P (10 μ M) + Zn^{2+} (10 μ M) + EDTA (50 μ M) ; e. P (10 μ M) + Zn^{2+} (10 μ M) + EDTA (50 μ M) + Zn^{2+} (50 μ M) ; f. P (10 μ M) + Zn^{2+} (10 μ M) + EDTA (50 μ M) + Zn^{2+} (100 μ M)



3.5. Interference Studies from other Metal Ions and Anions

The influence of common metal cations and anions on the identification of Zn^{2+} by P was further investigated, as shown in Fig.6. In addition to HCO_3^- , CO_3^{2-} and Ac^- , the other ions interfered with the fluorescence intensity of P- Zn^{2+} . The anti-interference ability of the P- Zn^{2+} system was low in the presence of most ions, so it is necessary to further optimize the structure of P to be used as a reference for the follow-up research.

Fig-6. Spectral response of probe P (10 μM) for identifying Zn^{2+} (10 μM) in the presence of different metal cation (50 μM) in ethanol (left) and anions (50 μM) (right).



4. Conclusions

In summary, we have presented a new pyrene-based Zn^{2+} probe. It exhibited a clear Zn^{2+} -induced change in the intensity at 522 nm. Thus, we expect that this strategy will serve as a practical tool for environmental samples analysis and biological studies.

5. Acknowledgment

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