

Research of a Novel Fluorescence Probe for Cu²⁺ and Its Applications in Environmental Analysis

Tian-tian SU^{1,2,a}, Yan LIU^{1,2,b,*} and Yi-min ZHU^{1,2,c}

¹Collaborative Innovation Center for Vessel Pollution Monitoring and Control, Dalian Maritime University, Dalian 116026, China

²College of Environmental Science and Engineering, Dalian Maritime University, Dalian 116026, China

^a1165981396@qq.com, ^bLiuyan07@dmlu.edu.cn, ^cntp@dmlu.edu.cn

*Corresponding author

Abstract—In this paper, a novel fluorescence probe based on Rhodamine(RB), which can be used in the quick detection of Cu²⁺ in environment, was synthesized. It was found that the addition of Cu ions led to a significantly enhanced fluorescence, while other common metal ions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Co²⁺, Ni²⁺, Ag⁺, Ba²⁺, Zn²⁺, Fe³⁺ and Fe²⁺ induced no or much smaller fluorescent spectral changes. The selective fluorescence enhancement of RN attributed to the spirolactam ring being opened upon the addition of Cu²⁺ to RN, and a highly delocalized conjugated structure of RN was formed.

Keywords- Cu²⁺, Rhodamine, Fluorescent probe

I. INTRODUCTION

Cu ions, as the third most abundant transition metals in nature, play important roles for both environmental and biological systems. The excessive Cu²⁺ is harmful to the ecological environment and the human body, so the detection of Cu ions has important significance to the research of life science and environmental science. In recent years, the detection methods of Cu ions are various, compared to instrumental methods such as mass spectroscopy (TCP-AES, ICP-MS) or inductively coupled plasma atomic emission, atomic absorption spectroscopy (AAS), etc, the fluorescent probes have been evaluated as the powerful tools to detect Cu²⁺ due to their several outstanding advantages such as the high sensitivity and selectivity, real-time detection and easiness of manipulating.

The fluorescence analysis method has been developed rapidly, the current main detection method is direct method, indirect method, synchronous fluorescence spectroscopy, derivative fluorescence spectrometry, three-dimensional fluorescence spectroscopy, solid surface fluorescence, fluorescence dynamic chemical analysis, the following is a brief introduction and focuses on indirect determination.

Fluorescence probe of Cu²⁺ mainly has two kinds of fluorescence, quenching probe and open loop fluorescence probe, but the quenching probe is easy to be disturbed by other ions and is not conducive to the output of high flux signal in detection, so the research value and practical value of the open loop fluorescence method of rhodamine derivatives. This paper adopts open loop fluorescence detection method, its principle is a lone pair of electrons N synthesis a coordination bond to produce a rose and a strong

fluorescent amine on the probe molecule Cu²⁺ empty orbit and lactam structure was destroyed in a spiral.

This rhodamine fluorescence probe detection applied to waste water solution. Intends to carry out the following aspects of the work: Preparation of rhodamine fluorescent probes and make optimization of detection conditions; fluorescence analysis method was established for the determination of related ions.

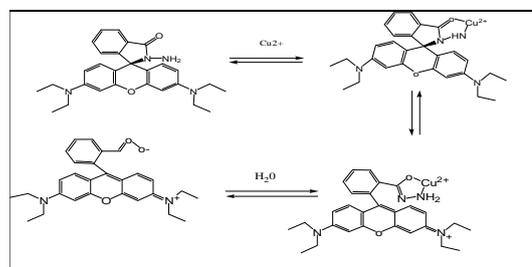
II. EXPERIMENTAL

A. Materials and Instruments

All solvents were commercially purchased and dried according to the standard procedures before use. Instrument: ls50b fluorescence spectrophotometer (Perkin Elmer); rotary evaporator (IKA RV10); magnetic stirrer; 85-2 type strong magnetic stirrer, zf-20d box type UV analyzer, DLSB low temperature cooling circulating pump, pH, column chromatography silica gel column, double beam UV visible spectrophotometer. ¹H NMR spectra were recorded on a Bruker AN-400 MHz in-instrument for solutions in d₆-Dimethyl Sulfoxide (DMSO), using tetramethylsilane as an internal reference.

B. Synthesis and Structure of Probe

The closed loop helix structure derivatives based on RB, its molecular formula is C₂₈H₃₂N₄O₂(RN), the structure and detection principles shown in the scheme 1. This probe in the synergy of Cu²⁺ and the carbonyl makes the probe hydrolysis and produce fluorescence for detection, the detection principle is shown in scheme 1.



Scheme 1. Reaction mechanism and the structure of RN probe.

C. Optimum Reaction Conditions of Fluorescent Probes

The environmental factors mentioned here mainly include: pH, temperature, solvent, paramagnetic, and so on. For the following sets of experiments to optimize the reaction conditions: optimal detection time; the choice of the best solvent; the choice of the best ratio of ethanol (20min) or acetonitrile (5 Min) as the solvent in the same reaction time contrast of fluorescence intensity of two solvents in the best ratio.

D. Cu²⁺ Fluorescence Detection Performance of Probe

Fluorescence selectivity of probe molecules on different metal cations; influence of metal interfering cations on the recognition of Cu²⁺; influence of metal interfering cations on the recognition of Cu²⁺ by the sequence of the addition of cations.

E. Complexation Ratio and Complexation Constant

The total concentration of fixed Cu²⁺ and RN unchanged, determination of luminous intensity solution by changing the relative concentrations of Cu²⁺ and RN, and then through the relationship between the solution composition and luminescence intensity of the composition of the obtained complexes generated in solution.

F. Establish Regression Equation and Applied to the Detection of Real Water Samples

The scattered points of different concentrations of Cu²⁺ and fluorescence intensity were plotted in the coordinate system, draw the standard regression curves.

III. RESULTS AND DISCUSSION

A. Synthesis of Probe

After a few steps to get the pink crystal, that is the probe RN, ¹H NMR characterization of RN (CDCl₃): (PPM) = 1.16 (T, 12, NCH₂CH₃), 3.33 (Q, 8, NCH₂CH₃), 6.30-6.46 (P, 4, xanthen-H), 7.08 (T, 2, xanthen-H), 7.26 (D, 1, Ar-H, 7.43) (t, 2, Ar-H), 7.94 (T, 2, Ar-H). (see Appendix 1).

B. Optimum Reaction Conditions of Fluorescent Probes

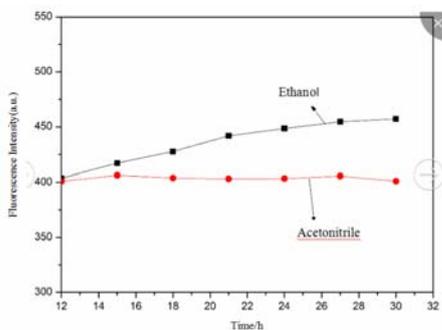


Fig. 1. Influences of solvent on the fluorescence spectra of ethanol and acetonitrile. (The pH was modulated by adding 1 M HCl or 1 M NaOH in HEPES buffers to 7.2.)

From the above experimental results and analysis can be drawn, in the RN probe for Cu²⁺ detection, using acetonitrile

as the solvent is better than ethanol, it can reach about 5 Min reaction equilibrium, make the reaction more time-saving. The volume ratio of acetonitrile and buffer solution was 3:1, the ethanol was 1:3. However, considering the negative effects of the toxicity of acetonitrile and the detection sensitivity, acetonitrile is an order of magnitude higher than that of ethanol. In the follow-up experiments and routine detection, acetonitrile was used as the probe solvent, and the buffer solution volume ratio is 1:3-3:1. See Figure 1,2.

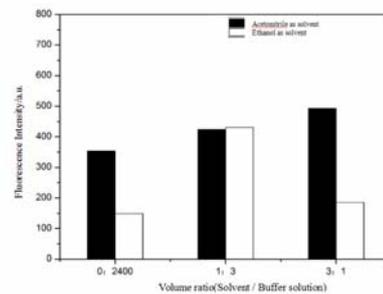


Fig. 2. Fluorescence intensity under different ratio of solvent (Ethanol / Acetonitrile) and buffer solution (v/v, PH=7.2).

C. Complexation Ratio and Complexation Constant

From the relation diagram (Figure 3) of the fluorescence luminescence maximum of RN and Cu²⁺ complexation with different proportions. we conclude that RN and Cu²⁺ in the ratio of 1 to 1, the fluorescence maximum, suggesting that the complexation ratio of probe to Cu²⁺ was 1 to 1. The stability constants of the interaction between Cu²⁺ and RN hydrate were calculated by using Origin to calculate the linear fitting method. The stability constant is 1.51 × 10⁴.

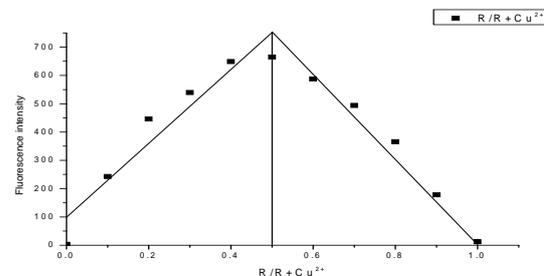


Fig. 3. The fluorescence intensity in different concentration ratio of RN and Cu²⁺ in acetonitrile solution (1:1, v/v, [Cu²⁺]/[Cu²⁺]+[RN]).

D. Cu²⁺ Fluorescence Detection Performance of Probe

The data is plotted as a spectral figure 4, we can also see that the Ag⁺ of the metal cations in the response is slightly higher than other cations, but the response to Cu²⁺ is still very low, it can be ignored. The interference of metal cations have certain effects on the molecular recognition probe Cu²⁺, different levels will make the fluorescence intensity decreased, but compared with only adding interference of metal cations, the response strength is still strong, so that the probe molecules in mixed metal ions in solution and the

detection of Cu^{2+} is still valid, is an effective Cu^{2+} enhanced fluorescence probe.

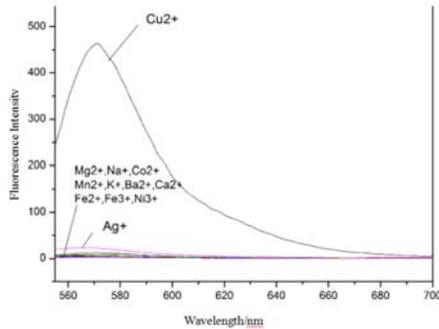


Fig.4. Fluorescence spectra of RN (10 IM) in an acetonitrile (3:1, v/v, 1 mM, pH 7.2) solution in the presence of different metal cations. The excitation wavelength is 574 nm.

Effect of metal interfering cations on the recognition of Cu^{2+} by the sequence of the addition of cations: The fluorescence intensity of adding interfering ions after adding Cu^{2+} were significantly lower than that of mixing after adding, this further proved that Fe^{3+} , Fe^{2+} could react and produce static quenching reaction of the fluorescence intensity decreased with RN molecules. The molecular probe detection reaction should be the interference of metal cations and Cu^{2+} after mixture were measured, this can avoid the fluorescence quenching effect of some metal cations to the maximum, but also more in line with the actual water samples.

E. The Establishment of Regression Equation

From the measurement of the standard regression curve can be seen, with the increase of the volume of the Cu^{2+} , the Cu^{2+} concentration in the solution gradually increased, the fluorescence intensity is also increasing, and basically in line with the linear relationship, draw the square of the correlation coefficient after the regression curve $R^2=0.988$, the regression equation obtained by this experiment is ideal, it can be used for the detection of real water samples. Equation parameters are shown in the figure 5.

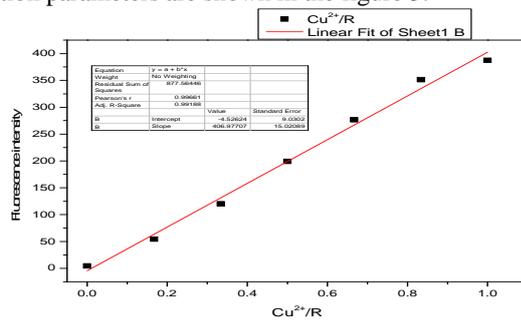


Fig.5. Fluorescence response of RN in the presence of increasing concentration of Cu^{2+} (from up to down the concentration of Cu^{2+} is 0, 0.2, 0.4, 0.6, 0.8, 1.0) in an acetonitrile (3:1, v/v, pH 7.2) solution.

F. Determination of Real Water Samples

In the above experiments, the solution was used as the reaction medium to investigate the fluorescence response of

the probe to Cu^{2+} . On this basis, the performance of the probe for Cu^{2+} detection in the real environmental water samples was further investigated. The samples was collected from a factory in Dalian, the probe was added to the samples to measure the luminescence intensity after diluted, and then the Cu^{2+} content was obtained. This method can be used to determine the recovery rate of Cu^{2+} in the 90.9%-99.8%, and has a smaller detection deviation (<3.1%), which indicates that the probe can be used for the accurate determination of Cu^{2+} content in the actual environmental samples.

TABLE.1. DETECTION of Cu^{2+} IN REAL WATER SAMPLES WITH SYNTHETIC PROBES.

Waste water	Added (M)	Found (M)	CV (% , n=3)	Recovery (%)
	0.00	3.74×10^{-8}	0.61	-
Sample 1	2.00×10^{-8}	5.63×10^{-8}	0.30	94.5
	1.00×10^{-7}	1.28×10^{-7}	0.60	90.9
	1.50×10^{-6}	1.54×10^{-6}	3.10	99.8

IV. ACKNOWLEDGMENT

Financial support from the National Natural Science Foundation of China (No. 21477011) and Higher Education Development Fund (for Collaborative Innovation Center) of Liaoning Province China (No.20110216006) are gratefully acknowledged.

V. REFERENCES

- [1] Gonçalves M S T. Fluorescent labeling of bio-molecules with organic probes[J]. Chemical reviews, 2008, 109(1): 190-212.
- [2] Bauer A J, Stockwell B R. Neurobiological applications of small molecule screening[J]. Chemical reviews, 2008, 108: 1774-1786.
- [3] Ametamey S M, Honer M, Schubiger P A. Molecular imaging with PET[J]. Chemical reviews, 2008, 108: 1501-1516.
- [4] H. Goto, Y. Hasegawa, and M. Tanaka, "Efficient Scheduling Focusing on the Duality of MPL Representatives," Proc. IEEE Symp. Computational Intelligence in Scheduling (SCIS 07), IEEE Press, Dec. 2007, pp. 57-64, doi:10.1109/SCIS.2007.357670.
- [5] Tang B, Cui L J, Xu K H. A Sensitive and Selective Near - Infrared Fluorescent Probe for Mercuric Ions and Its Biological Imaging Applications[J]. Chem-Bio-Chem, 2008, 9: 1159-1164.
- [6] Taki M, Iyoshi S, Ojida A. Development of highly sensitive fluorescent probes for detection of intracellular copper (I) in living systems[J]. Journal of the American Chemical Society, 2010, 132: 5938-5939.
- [7] Sun Z N, Liu F Q, Chen Y. A highly specific BODIPY-based fluorescent probe for the detection of hypochlorous acid[J]. Organic letters, 2008, 10: 2171-2174.
- [8] Koide Y, Urano Y, Kenmoku S. Design and synthesis of fluorescent probes for selective detection of highly reactive oxygen species in mitochondria of living cells[J]. Journal of the American Chemical Society, 2007, 129: 10324-10325.
- [9] Fujikawa Y, Urano Y, Komatsu T. Design and synthesis of highly sensitive fluorogenic substrates for glutathione S-transferase and application for activity imaging in living cells[J]. Journal of the American Chemical Society, 2008, 130: 14533-14543.

